The Effect of Bovine Colostrum Supplementation on Fitness, Muscle Mass, Inflammation and Immune Function, During the Regular Season, in Rugby Players

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Abstract

Bovine colostrum is the first milk secreted by cows after calving. Even though bovine colostrum has similar protein content as regular milk, it contains greater levels of anti-microbial substances and growth factors. Intense training may compromise the immune system; therefore, colostrum has potential to improve exercise performance by preventing immune system dysfunction that is common during periods of intense training.

PURPOSE: The purpose was to determine the effect of 8 weeks of bovine colostrum supplementation in comparison to soy protein supplementation, on rugby players': body composition, strength, endurance performance, vertical jump height, and IgA, IL-6, IL-1β and C reactive protein levels, during the regular rugby union season.

METHODS: Twenty-nine rugby union players (n=3 female) were randomized into two groups (double blind); Group 1 received 38g/d of protein from bovine colostrum, and Group 2 received 38g/d of protein from soy. Before and after the 8 weeks of supplementation, participants were evaluated for IgA, IL-6, IL-1β and C reactive protein levels from salivary samples, body composition using dual energy X-ray absorptiometry, muscle thickness of their quadriceps and biceps using ultrasound, bench press and leg press strength, vertical jump height, and aerobic fitness using the Leger maximal multistage 20-m shuttle run field test.

RESULTS: There were no differences between groups over time in all variables other than vertical jump height. There was a significant group x time interaction for vertical jump (p=0.002) with the colostrum group (+0.9cm) increasing more than the soy group (-0.5cm).

CONCLUSION: BC supplementation in rugby union players during the regular season did not affect body composition, salivary immune markers level and fitness components (excluding vertical jump height) in comparison to soy protein supplementation. Bovine colostrum supplementation may enhance leg power to a greater extent than soy protein supplementation.

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List of Abbreviations

1RM: One repetition maximum

BC: Bovine colostrum

CMJ: Counter movement jump

CRP: C reactive protein

DXA: Dual energy X-ray absorptiometry

IgA: Immunoglobulin A

IL-1β: Interleukin 1-beta

IL-6: Interleukin 6

VO_2max: Maximal oxygen consumption,

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1. Scientific Framework

1.1 Introduction

1.1.1 Bovine Colostrum

Bovine colostrum (BC) is the first milk secreted by cows after calving. Even though BC has similar protein content as regular milk, it contains greater levels of anti-microbial substances and growth factors (Donavan and Odle 1994; Mero et al. 2002). The main growth factor in BC is insulin-like growth factor-1 (Francis et al. 1988) which stimulates growth of muscle tissue (Tomas et al. 1991) and is important in maintaining muscle mass and function in adults (Borst et al. 2001). One of the main anti-microbial substances in BC is immunoglobulin A (IgA). IgA levels in BC are approximately a hundredfold higher than in regular milk (Mach and Pahud 1971). Other anti-microbial substances that are much higher in BC than in regular milk are proteins and peptides such as lysozyme and lactoferrin (Korhonen 1977; Shing et al. 2009) which are essential for proper immune system function.

Previous studies have shown improvements in body composition (Antonio et al. 2001), power (Buckley et al. 2003; Hofman et al. 2002), and strength (Duff et al. 2014), when BC supplementation was used during a resistance training program. The mechanism through which BC acts to benefit performance and body composition, remains unclear.

1.1.2 Rugby

Rugby is an intermittent contact team sport with periods of high-intensity activity (running, tackling) and low-intensity recovery (walking, jogging, and standing) taking place over two 40-minute halves with a 10-minute half time interval. The physiological demands of rugby union are complex when compared with individual sports (e.g. running, cycling, and swimming).

Mean heart rates of 82% to 84% of maximum heart rate have been reported for elite players during competitive matches (Twist and Highton 2013). Moreover, players were shown to

spend a considerable percentage (30-44%) of total match-play in high-intensity activities with the mean intensity of semi-professional matches reported to be 81% VO_2max (Gabbett 2007). A high VO_2max facilitates the repetition of high-intensity efforts, and is related to the distance covered, level of work intensity, number of sprints, and involvements with the ball (Duthie et al. 2003). Duthie et al. (2003) concluded that the energy contributions during the work periods in intermittent team games, such as rugby, are mainly anaerobic. The mean blood lactate concentrations during competition for semi-professional rugby league, was found to be 7.2 mmol/L (Coutts et al. 2003).

Rugby performance requires high levels of muscular strength and power for success, particularly in the execution of tackles, explosive acceleration, scrummaging (when players are packing closely together with their heads down and attempting to gain possession of the ball), and forceful play during rucking (a ruck is formed when one player or more, from each team, bind onto each other with the ball on the ground between them) and mauling (a maul is formed when the ball carrier is being held by the opponents, and one or more of his team mates, bind to him. The mean force that one team produces during scrummaging, ranges from 6210 to 9090 N (O'Gorman et al. 2000). These findings led Twist and Highton (2003) to conclude that rugby places considerable physiological demands on both the aerobic and anaerobic energy systems. The demands required during a rugby game can lead to fatigue, tiredness and decrements in muscle performance and function. Gabbett (2005) and Schneider et al. (1998) found that teams' in-season training programs, emphasize skills (catching, ball-carrying, etc.), speed, agility, and aerobic training, while neglecting strength training. This may increase the loss of lean tissue mass and strength, resulting in a greater injury risk (Gabbett 2005).

Understanding the nutritional needs of rugby players is very important in order to optimize performance and recovery (Casiero 2013). The amount of carbohydrate that rugby players require is between 5 to 8 g/day, per kilogram of body mass (Casiero 2013; Kreider et al. 2010). In addition, it is also recommended that the source of 20% to 30% of rugby players' total daily caloric intake, should be fat (Venkatraman and Pendergast 2000). Rugby players also require more protein than the average population, in order to support muscle growth and improve recovery (Casiero 2013). It was recommended that rugby players should consume twice the recommended daily allowance of protein (1.5- 2 g/day per kg, instead of 0.75-1 g/day per kg), in order to prevent a negative nitrogen balance (Chesley et al. 1992; Lemon et al. 1992;

Tarnopolsky et al. 1988), which may impair performance and slow recovery (Leutholtz and Kreider 2001). The type of protein is also important, as casein and whey proteins for example display different rates of digestion and absorption, and are beneficial for decreasing whole body catabolism (Boirie at al. 1996). Hence, Casiero (2013) suggested that rugby players should be encouraged to eat not only an adequate amount of protein, but also protein from quality sources such as chicken, milk, eggs and fish.

Supplements also play a role in rugby players' nutrition. One of the main supplements being used is creatine monohydrate, which has been shown to increase muscular endurance in rugby players (Chilibeck et al. 2007), and high intensity exercise capacity and lean muscle mass in other athletes (Kreider et al. 2010). Another popular supplement among rugby players is caffeine, which enhances sport performance when consumed in low to moderate doses (3 to 6 mg per kg) (Goldstein et al. 2010). Caffeine is also beneficial for high intensity exercise of prolonged duration in team sports (Schneiker et al. 2006).

Intense training, as in rugby, can also compromise the immune system. Neutrophil function, immunoglobulins concentrations and Natural killer cell number are all suppressed during prolonged periods of intense exercise training (MacKinnon 2000). Therefore, BC has potential to improve fitness, possibly by preventing immune system dysfunction that is common during periods of intense training (Shing et al. 2009).

1.2 Literature Review

The first study to investigate the effect of BC supplementation on exercise performance was Mero et al. in 1997. Since then, research has investigated the ability of BC supplementation to improve body composition, strength, endurance, anaerobic performance, power, and immune system functioning. In addition, researchers are also trying to determine mechanisms for these improvements.

1.2.1 Body Composition and Strength

The evidence, in regards to the effect of BC supplementation on body composition and strength, is contradictory. Two studies, using doses of 20-60 g/day during 8 weeks of training

found no improvement in strength with BC supplementation: Antonio et al. (2001) used a dose of 20 g/day in 22 active participants, who followed a 3 days a week resistance training program, and Buckley et al. (2003) used a dose of 60 g/day in 51 "active" males, who followed a 6 days a week alternate resistant training and plyometric training program. On the other hand, two other studies found that BC supplementation with a dose of 60 g/day improved strength after 8 or more weeks of training: Kerksick et al. (2001) used a population of 49 "resistance trained" participants who followed a 4 days per week resistance training program (for 12 weeks), and Duff et al. (2014) used a population of 40 older adults who followed a 3 days per week resistance training program (for 8 weeks).

A similar inconsistency can be found in regards to the effect of BC supplementation on body composition. While Duff et al. (2014) did not find any significant differences in body composition (measured by Dual-energy X-ray absorptiometry) between their BC group and the control (whey) group (using a dose of 60 g/day), Antonio et al. (2001) found a significant difference in lean tissue mass, between their BC group and a control group (in favor of the BC group) even though they used a lower dosage of 20 g/day. In addition, Brinkworth et al. (2004) found a significant change in lean tissue mass (greater cross sectional area in the upper arm muscles) in favor of BC supplementation of 60 g/day, versus placebo, after 8 weeks of supplementation and strength training for 4 days a week. One possible reason for this inconsistency might be the fact that the participants in the Duff et al. study were elderly, while the participants in the other studies were young and physically active, and thus, may be more able to a change body composition.

To sum up, from the limited amount of previous work, there is no conclusive evidence for BC supplementation to affect body composition or strength. In addition, it seems that higher doses of BC are required in order to notice an effect. Using a higher dose of 60 g/day of BC would help in maximizing and detecting the effect, if there is one. On the other hand, higher doses of more than 60 g/day of BC may decrease compliance, as it will require participants to ingest bigger serving sizes, and/or increase the ingesting frequency.

1.2.2 Power

Power ("explosiveness") is the rate at which mechanical work is done (i.e. the product of force and velocity) and it is a very important quality in athletes' performance, especially in sports such as football, rugby, weightlifting, etc. (Winter and Fowler, 2009). BC supplementation in athletes, at a dose of 60 g/day, for 8 weeks during training improved vertical jump height (an indirect measurement of power) and peak cycle power, in comparison to supplementation with placebo or whey (Leppäluoto et al. 2000; Buckley et al. 2003). However, while these findings might suggest that BC is beneficial to power activities, Leppäluoto et al. (2000) did not report the details of training and diet control (which might be confounders), and what is more, another study involving a similar dosage (60 g/day) and duration (8 weeks), showed no improvement in vertical jump performance in athletes who received BC supplementation (Hofman et al. 2002). Therefore, limited conclusions can be drawn in regards to the effect of BC supplementation on power, as prior evidence is far from being conclusive.

1.2.3 Anaerobic Performance

Anaerobic glycolysis results in the production of H^+ and lactate. Buffer capacity is the ability to bind free protons (i.e. to buffer H^+), and offset reductions in pH during exercise; hence, given the strong association between acidosis and muscular fatigue, buffer capacity is an important attribute for maintaining anaerobic performance (Parkhouse and McKenzie 1984). The main buffers of H^+ come from skeletal muscle and include protein, inorganic phosphate, and phosphocreatine. Other components in blood including haemoglobin, bicarbonate and plasma proteins also buffer H^+. Brinkworth et al. (2002) examined whether BC supplementation of 60 g/day (vs. whey) could enhance the buffering of H^+, in response to a 9-week training program. Two incremental rowing tests on a rowing ergometer (consisting of 3 x 4 minute submaximal workloads and a 4 minute maximal effort, each separated by a 1 minute recovery) were used to assess performance, before and after the completion of the supplementation period. Buffering capacity was estimated from the differences in the blood lactate levels and the blood pH levels, which were taken at the end of each workload during the testing period. It was found that buffering capacity was significantly increased after BC supplementation versus whey. From the findings of a following study (Brinkworth and Buckley, 2004), it was determined that the

difference is the result of enhanced muscle buffering capacity, and not haemoglobin levels, plasma bicarbonate levels, or plasma buffering capacity in general.

Other studies measured anaerobic performance in athletes in a more practical way, measuring performance variables, in the athletes' "natural" environment. The findings from these studies seem to contradict each other. While Hofman et al. (2002) found that a supplementation of 60 g/day BC for 8 weeks significantly improved repeated sprint running performance (in comparison to placebo), Shing et al. (2006) found no improvement in a time-to-fatigue test at 110% of ventilatory threshold, between BC and placebo in 29 highly trained male road cyclists. It is important to note that Shing et al. (2006) used a lower dosage of 10 g/day of BC, which may not be sufficient to produce a measurable effect. On the other hand, another study, using a higher dosage of 60 g/day of BC also found no significant difference (vs. placebo) in cyclists' anaerobic performance (Buckley et al. 2003).

In sum, it appears that there is inconsistency in the evidence for the positive effect of BC supplementation on anaerobic performance. Also, if a positive effect of BC supplementation on anaerobic performance does exist, the mechanism for this effect is yet to be determined.

1.2.4 Endurance Performance

Endurance performance (i.e., aerobic fitness) refers to the use of oxygen to adequately meet energy demands during exercise via aerobic metabolism. Endurance performance is crucial in sports such as long distance running/walking, long distance swimming, cycling, soccer, and rugby. One possible mechanism for the potential positive effect of BC on aerobic fitness is an improved muscle glycogen resynthesis (Shing et al. 2009). Although increased muscle glycogen levels during normal training do not improve endurance performance (Hawley et al. 1997), during repeated days of high-intensity exercise, increased muscle glycogen levels may prevent and delay fatigue (Kavouras et al. 2004; McInerney et al. 2005). Eight weeks of BC supplementation in dosages ranging from 10-60 g/day improved aerobic performance (in comparison to whey or placebo): Coombes et al. (2002) assessed 42 professional cyclists who consumed either 20 or 60 g/day (significant differences were found with both doses vs. whey), while Shing et al. (2006) used 29 professional cyclists who consumed 10 g/day of BC for eight weeks (vs. placebo). BC supplementation may improve glycogen levels by enhancing the activity

of some of the enzymes involved in gluconeogenesis (and therefore perhaps synthesis of glycogen), as it was shown that colostrum feeding in calves enhanced activity of the rate-limiting enzymes for gluconeogenesis, pyruvate carboxylase and phosphoenolpyruvate carboxykinase (Hammon et al. 2003).

Another possible mechanism for the positive effect of BC supplementation on aerobic performance may be that it prevents the decrease in testosterone concentration during long-duration exercise and therefore improves protein synthesis or prevents protein catabolism during endurance training (Shing et al. 2013). In addition, some of the growth factors found in BC may enhance nutrient uptake from the intestine; this has been suggested as another possible mediator to improve adaptation to intense aerobic training (Coombes et al. 2002). BC may also have direct effects on cardiovascular functioning: eliciting lower heart rate, higher cardiac output, and elevated parasympathetic indices of heart rate variability (i.e., increased intervals between two consecutive R waves in the ECG) (Shing et al. 2013).

Overall, it appears that BC supplementation is likely to improve endurance performance. However, the mechanism by which BC acts to improve endurance performance is yet to be definitively determined. While the above- suggested mechanisms (improved muscle glycogen resynthesis, enhanced nutrient absorption) may be possible, alterations in respiratory exchange ratio (alterations in the oxidation of carbohydrate vs. fat), lactate threshold or insulin like growth factor 1 (IGF-1) levels have been ruled out (Shing et al. 2009).

1.2.5 Immune Function

Intense exercise suppresses immunity for several hours (Nieman 2002). Hence, athletes that perform high intensity training are at a high risk for over-training syndrome (Halson et al. 2002; Halson et al. 2003; Mackinnon 2000) and upper respiratory tract infections (Mackinnon 2000; Fitzgerald 1991). Over-training syndrome is a neuroendocrine disorder characterized by poor performance in competition, inability to maintain training loads, persistent fatigue, reduced catecholamine excretion, frequent illness, disturbed sleep and alterations in mood state (Mackinnon 2000). It is estimated that, at any given time, between 7 and 20% of all athletes may exhibit symptoms of overtraining syndrome. Overtraining syndrome can be due to excessively large volumes of training, without adequate rest and recovery (Mackinnon 2000).

There are a large number of markers of immune function that one can measure from blood or saliva samples. The markers that were chosen for assessment in this thesis are reviewed in the following section.

1.2.5.1 Immune Function Markers

1.2.5.1.1 Immunoglobulin A (IgA)

Immunoglobulin A (IgA) plays key roles in immune protection while being a major serum immunoglobulin and the main antibody class in the external secretions that immerse mucosal surfaces (Woof and Kerr 2006). An immunoglobulin (also known as an antibody), is a Y-shape protein produced by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses.

A substantial amount of energy is expended in the production of IgA. This amount of energy exceeds the amount of energy that is required for the production of all the other antibody classes combined. This may suggest that the benefits provided by IgA, in terms of immune defence, must be substantial (Woof and Kerr 2006).

IgA protects the vast surface area occupied by mucosal surfaces, such as the linings of the respiratory, gastrointestinal, and genitourinary tracts. As the major class of antibody present at these sites and by being the first line of defence against many invading pathogens, IgA is considered very important to maintain proper function of the innate immune system (Woof and Kerr 2006). This system is an important subsystem of the overall immune system that comprises the cells that defend from infection. Unlike the adaptive immune system, it does not give long-lasting immunity to the host, but an immediate defense against an infection.

It seems that the very high concentration of secretory IgA in human (and other mammals) colostrum and milk, strongly suggests that IgA must play a crucial role in the passive immune protection of the newborn (Woof and Kerr 2006).

1.2.5.1.2 C-reactive protein (CRP)

C-reactive protein (CRP) was discovered by Tillett and Francis in 1930 as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of

pneumococcus. CRP interacts with the complement system to activate an immunologic defense mechanism. The complement system is a group of proteins that move freely throughout the bloodstream. They work with the immune system and play a role in the development of inflammation. Synthesis of CRP occurs in hepatocytes and is regulated primarily by the proinflammatory cytokines: interleukin-1β, interleukin-6, and tumor necrosis factor-α (Ingle and Patel 2011).

CRP has a normal range in the blood of < 2 mg/L. In populations with illnesses such as rheumatoid arthritis or sepsis, the concentrations can increase up to 300 mg/L (Ingle and Patel 2011). In athletes, CRP levels are increased when overtraining syndrome, or tissue injury have occurred (Smith 2004).

1.2.5.1.3 Interleukin 1 beta (IL-1β) and Interleukin 6 (IL-6)

Interleukin 1 beta (IL-1β) and Interleukin 6 (IL-6) are pro-inflammatory cytokines. The injection of them (in order to mimic secretion) into animals or humans, was shown to produce the acute phase inflammation response; these cytokines facilitate an influx of lymphocytes, neutrophils, monocytes and other cells, which participate in the clearing of antigens and healing of tissue (Ostrowski et al. 1999).

Both IL-1β and IL-6 levels are elevated in the circulation after an acute exercise. Although IL-1β is elevated for only a few hours post exercise, it has also been proposed to mediate anabolic and catabolic processes that can last for several days (Pedersen et al. 2004). The IL-6 also acts as an energy sensor, being dependent on the glycogen content in the muscle; it is released from contracting muscles in high amounts and it induces lipolysis (Pedersen et al. 2004).

1.2.5.2 Bovine Colostrum, Exercise, and Immune Function

There is evidence that several immune parameters are suppressed during prolonged periods of intense exercise training. These include neutrophil function, serum and salivary IgA concentrations, and natural killer cell number (Mackinnon 2000). It has also been proposed that

the physical stress of intense exercise training initially causes elevation of stress hormone levels, such as CRP (Smith 2004), catecholamines and glucocorticoids (Urhausen et al. 1995).

Cytokines have also been proposed as mediators of overtraining syndrome. Repetitive, high volume exercise with inadequate rest causes injury (microtrauma) to joints, muscles and connective tissue. This injury in turn activates monocytes to produce and release inflammatory cytokines such as IL-1b, IL-6 and TNF-a (a cytokine that is involved in an acute systemic inflammation), initiating chronic systemic inflammation and suppressed immune function (Shing et al. 2009).

Although lymphocytes levels are not different between rest and overtraining syndrome, lymphocytes may be activated to a greater extent by periods of intense exercise training (Fry et al. 1992; Rhind et al. 1994). For example, expression of high-affinity IL-2 receptor was higher on cells in distance runners compared with matched non-runners (Rhind et al. 1994). In addition, a significant increase in low-affinity IL-2 receptor expression has been observed in cells obtained over 10 days of intense running training that caused overreaching, despite no changes in lymphocyte levels (Fry et al. 1992).

A depressed immune system is associated with an increased risk of upper respiratory tract infection. One potentially beneficial effect of BC on immune function may be reduction in upper respiratory tract infection symptoms. It is thought that an increase in salivary IgA affords greater protection against upper respiratory tract infection (Shing et al. 2009). However, there is no ideal single marker to measure immune modulation following a nutrition intervention (Gleeson 2005).

In infants, colostrum stimulates immune development through lymphoid tissue in the oropharynx and the gut. The absorption of the immunologic factors by the oral mucosa stimulates the immune system systemically, and promotes the mucosal differentiation in the gut, developing the protective gut immune barrier (Gephart & Weller, 2014). Interestingly, in preterm infants who cannot tolerate enteral feedings, and receive an oropharyngeal administration of colostrum (an intervention in which a small amount of colostrum is placed directly onto the oropharyngeal mucosa in the buccal cavity for absorption. This does not involve the infant's swallowing of milk), the immune factors in colostrum still interact with lymphoid tissues in the oropharynx, and still stimulate the immature neonatal immune system. This finding may suggest that in absorption of immunologic factors from colostrum take place in other mucosal tissues, in addition to the gut (Lee et al. 2015).

A number of studies examined the effect of BC supplementation on IgA concentration. Some of these studies found no significant difference in IgA levels after supplementing with BC (Mero et al. 1997; Shing et al. 2007; Shing et al. 2013). However, Mero et al. (2002) and Crooks et al. (2006) who used 20 g/day for 2 weeks and 26 g/day for 12 weeks respectively, found a significant difference in IgA levels after supplementing with BC. The reason for this contradiction in findings could be that Mero et al. (1997) conducted their first study for 8 days only, a period of time which may be too short in order to present a significant effect, and that Shing et al. (2007; 2013) used a dosage of 10 g/day only, which may not be enough to create a detectable change in IgA levels.

A few studies have also instigated the relationship between BC supplementation and upper respiratory tract infections. Brinkworth and Buckley (2003) found that the percentage of participants that had upper respiratory tract infections was significantly lower in a group who received BC (60 g/day for 8 weeks) compared to placebo. However, Crooks et al. (2006) found no significant difference in upper respiratory tract infections incidence, despite a significant increase in salivary IgA levels in the BC group (vs. whey).

Again, although some studies suggest that BC supplementation may be associated with increased levels of IgA levels, there appears to be discrepancies in the research on the effect of BC supplementation on immune function and the possible mechanisms.

1.2.6 Insulin-like Growth Factor-1 (IGF1)

IGF-1 is a hormone that plays an important role in childhood growth and continues to have anabolic effects in adults. Despite the fact that BC contains IGF-1, only one group of authors (out of more than a dozen) reported significant increases in IGF-1 levels after BC supplementation for 8 and 14 days (Mero et al. 1997; Mero et al. 2002). IGF-1 is usually degraded in the gastrointestinal tract, but it was suggested that some factors in BC may improve the absorption of IGF-1 by preventing its breakdown (Playford et al. 1993).

Normal IGF-1 levels for young adults are 14-48 nmol/L. The increase reported in the studies mentioned above, was approximately 5 nmol/L, while the amount of IGF-1 contained in the BC was 74 μg/day. At this dose, if 65% of IGF-1 was absorbed, the concentration of IGF-1 would only be expected to rise only by approximately 1.05 nmol/L. This suggests that the

increase in serum IGF-1 was probably due to an increase in endogenous production (Shing et al. 2009). Other studies with similar doses of BC and longer supplementation periods, have reported no significant changes in IGF-1 levels following 4–8 weeks of BC supplementation (Buckley et al. 2003; Coombes et al. 2002).

Given the fact that IGF-1 is usually degraded in the gastrointestinal tract, and that all studies but two (Mero et al. 1997; Mero et al. 2002) found no significant changes in the levels of IGF-1 after BC supplementation, it seems likely the possible positive effects of BC supplementation on body composition and/or fitness are not due to IGF-1 levels.

1.3 Purpose and Hypotheses:

The purpose of the study is to determine the effect of 8 weeks of bovine colostrum supplementation in comparison to soy protein supplementation, on rugby players': fitness, body composition, and IgA, IL-6, IL-1β and CRP levels, during the rugby season. We chose to focus on rugby union players, who undergo intense training programs during their playing season, as the physiological demands of rugby union are complex (all the components of fitness play a role; strength, power, anaerobic and aerobic fitness) in comparison to individual sports such as swimming, running, etc. This unique sport, with its many various physical demands, makes it possible to track and test multiple outcomes in the same study. In addition, intense training, as in rugby, can compromise the immune system. Therefore, rugby players would be a good population to examine the effect of BC on the immune system.

It was hypothesized that BC supplementation would result in greater anabolic effects in response to intense training which would translate into more favorable changes in lean tissue mass, strength, jump height and aerobic performance, compared to soy protein.

The secondary hypothesis was that BC supplementation would result in lower levels of salivary IL-6, IL-1b and CRP, and a higher level of salivary IgA, in comparison to soy supplementation.

2. Methodology

2.1 Study Design and Intervention

Twenty-nine rugby union players (n=3 women) were randomly assigned, by use of a computerized random number generator (1:1 allocation ratio), to receive 38g/day of protein from BC protein powder (63g of BC supplement) or soy protein powder (72g of soy supplement) which was used as control, for eight weeks during their rugby season.

The study was double blinded: researchers, participants, and all individuals conducting outcome assessments were unaware of group assignments. Both groups were provided with a 5 Litre container that included the supplement and a measuring scoop. Buckets were identical and the scoops were similar but different in size (volume). Blinding was accomplished using an individual, who was independent of the rest of the research team, to prepare the numbered buckets containing the supplement. BC was a heat-treated spray-dried, >25% IgG, commercially available product (trade-named Eterna Gold manufactured and marketed by the Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada). BC was derived from first day postpartum excess colostrum collected from Canadian dairy cows and is licensed by Health Canada as a natural health product for immune system and athletic support (Natural Health Product Number 80035324). Both supplements were flavourless, odorless, and had the same color, and texture.

BC dose chosen was based on the effective dose for increasing lean tissue mass during strength training of young participants (Antonio et al. 2001, Kerksick et al. 2007), and increasing muscle strength in older participants (Duff et al. 2013). Soy protein was used as a control supplement because it matched the nitrogen (i.e. protein content) and caloric content of BC without delivering the same bioactive molecules found in BC (Table 2-1). Participants were to consume the supplement 3 times a day, with one time before a workout and another time immediately after a workout.

During these 8 weeks, the players followed their regular season training routine, which consists of two 2-hour rugby practices per week and one 80-minute game. Participants also had

between two to three strength workouts a week. In order to control for training volume, players recorded their first and last week training regime and games (minutes) played. To assess dietary intake, three-day dietary records were collected at baseline and during the last week of supplementation, as three-day records were shown to produce the best agreement between reported and observed intakes (Crawford et al. 1994), and are considered to be an acceptable dietary assessment tool (Yang et al. 2010). Evaluation of nutrient intakes was derived from the US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, USDA National Nutrient Database for Standard Reference, Release 28, May 2016. This database is a major source of food composition data in the United States, and contains data on 8,789 food items and up to 150 food components.

Table 2-1. Nutritional content of BC and soy supplements.

	Content in the daily dosage of BC dry powder (60g)	Content in daily dosage of soy protein dry powder (72g)
Calories (kcal)	284	262
Fat (g)	9	1
Saturated fat (g)	5	0
Cholesterol (mg)	110	0
Carbohydrate (g)	13	25
Sugars (g)	5	11
Protein (g)	38	38
Calcium (mg)	190	224
Iron (mg)	1	12
Magnesium (mg)	151*	86
Phosphorous (mg)	919*	518
Potassium (mg)	587*	278
Sodium (mg)	151	72
Zinc (mg)	8*	3
Copper (µg)	70*	1
Manganese (µg)	21*	3
Vitamin B1 (µg)	185*	185
Vitamin B6 (µg)	80*	62
Vitamin B3 (µg)	68*	432
Vitamin B2 (µg)	926*	62

*Values were analyzed by the Saskatoon Colostrum Company, and were calculated from Kehoe et al. (2007) reported at 27.6% total solids and assuming 1.06 g/ml (Christiansen 2010).

2.2 Participants

Participants were recruited via presentations to individual rugby clubs in the city of Saskatoon, SK, Canada. Only players who were 18 years old or older were allowed to participate in the study. Players who had taken other nutritional supplements within the month prior to study initiation, and players with milk or soy allergies, were excluded. As part of our screening process, participants filled out a "Physical Activity Readiness Questionnaire" (i.e. PAR-Q+, Appendix A) form to ensure the physical testing will be safe for them. This form assesses participants for medical issues that may place them at risk from exercise testing (Chilibeck et al. 2011, Warburton et al. 2011). If determined to be at risk, players needed to acquire permission from their family physicians to participate in the study.

Sample size was based on the expected mean change in lean tissue (i.e. muscle) mass with BC supplementation (i.e. 1.5kg-2 kg) compared to control (i.e. 0kg-1.2 kg) with a standard deviation for this change of 0.5 to 1.0 kg (Antonio et al. 2001; Kerksick et al. 2007), an alpha of 0.05 and power of 0.8. Although the sample size calculation indicated that 10 participants per group (i.e. 20 in total) were required, nine additional participants were recruited to account for participants who might withdraw from the study.

Ethical approval for this study was obtained by the University of Saskatchewan's biomedical research ethics board. Participants were provided with a written and oral overview of the study, were given an opportunity to ask questions about the study, and were provided with an informed consent form (Appendix B) to read and sign.

2.3 Measurements and Dependent Variables

The primary outcomes for this study were the mean changes over time, between groups, for body composition and strength. Secondary outcomes were the mean changes over time, between groups, for vertical jump height, aerobic performance, muscle thickness, and the prevalence of upper respiratory tract infections. Measurements for all of these variables were obtained prior to the 8 weeks of supplementation (at time of randomization), and 1-3 days after the supplementation period.

2.3.1 Body composition

Body composition was assessed with dual energy X-ray absorptiometry (DXA) (QDR Discovery Wi; Hologic, Inc., Bedford, MD, USA) using QDR software for Windows XP (QDR Discovery, Hologic, Inc.). Fat mass was assessed from whole-body scans. Lean tissue mass was assessed from whole-body scans and also from the trunk, arms and legs regions. The coefficient of variation for lean tissue mass is 0.5%, and for fat mass is 3% (Chilibeck et al. 2013).

2.3.2 Strength

Strength was assessed by performing a one repetition maximum (1RM) on a leg press machine and a bench press machine with more than 15 minutes between exercises. The 1RM protocol consisted of a 5-8 repetition set with low weight and a 1-min break before the first 1RM attempt. Weight was then progressively increased and a 3-5min rest period was given between each subsequent 1-RM attempt. Subjects were permitted four to six attempts to determine their 1-RM value. This method was previously used in our lab and was described by Chrusch et al. (2001). During the leg press 1RM hands were placed on the seat handles the whole time, and the back and buttocks kept contact with the seat. A full repetition was only considered when legs were fully extended.

During the bench press 1RM hands were placed approximately shoulder width apart, feet were on the floor and the back and buttocks kept contact with the bench the whole time. A full repetition was only considered when elbows were fully extended. The coefficients of variation for repeated leg press and bench press strength assessments were 3.0% and 3.6%, respectively.

2.3.3 Vertical Jump Height

We estimated lower body power by a counter movement vertical jump (CMJ) height, using the Vertec device for the measurement. Participants stood barefoot and reached up with their arm as high as they could, while their heel was still touching the ground. This height was recorded as the initial height. Then, participants were given three attempts to jump vertically (with the assistance of an arm swing) as high as they could. The highest result from the three attempts was recorded. Then, the initial height was subtracted from the maximal height. The coefficient of

variation for repeated measures of this test was 1.0% (determined by repeated tests on the participants).

2.3.4 Aerobic Performance

Predicted VO_2max was assessed using the Leger maximal multistage 20-m shuttle run field test ("the beep test"), where players ran 20m distances at increasing velocity (done to the cadence of beeps on a Compact Disc) until exhaustion (Leger and Lambert 1982). A higher 20m stage completed indicates a greater aerobic fitness and a higher predicted VO_2max. Stages were converted to predicted relative VO_2max using a conversion table (Leger and Lambert 1982). The coefficient of variation for this test is 3.5%.

2.3.5 Markers of immune function

IgA, CRP, IL-6 and IL-1β levels were analyzed from saliva samples using enzyme-linked immunoabsorbent assays (ELISA) by Salimetrics, State College, PA (Appendix C).

Sample collection was done >60 minutes after eating a major meal. In addition, participants were also instructed not to consume alcohol for at least 12 prior to the sample collection, as acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, the participants rinsed their mouth thoroughly with water 10 minutes before the sample was collected. Saliva was collected from a passive drool through a short straw and into a polypropylene vial. After collection, samples were stored in a -20ºC freezer, in order to avoid bacterial growth and loss of immune markers in the specimen.

On day of assay, the samples were thawed completely, vortexed, and centrifuged at 3000 rpm for 15 minutes. Centrifuging removes mucins and other particulate matter which may interfere with antibody binding, leading to falsely elevated results. All samples were analyzed in duplicate and read in a plate reader at 450nm. The final value taken for each marker was the average of the two duplicates from the same sample, when coefficient of variation (CV%) <15. If the CV% between the duplicates was higher than 15, the samples were reanalyzed again. The average inter-assay precision (CV%) of 20 replicates for CRP, IL-1β and IL-6 is 5.9%, 2.5% and 4.8% respectively. The average inter-assay precision (CV%) of 8 replicates for IgA is 8.79%.

Details of the testing kits procedures can be found in Appendix C.

2.3.6 Muscle Thickness

Muscle thickness was measured using a B-mode ultrasound device (Aloka SSD-500, Tokyo, Japan). The muscle thickness sites included the quadriceps and biceps. The quadriceps site was land marked on the right leg at 70% of the distance down from the greater trochanter to the lateral epicondyle of the tibia. A tape measure was wrapped around the right leg at the 70% mark and was used to mark the reference point on the bulk of the vastus lateralis. Participants were placed on a table in a seated position with their right leg extended and relaxed.

The biceps site was on the bulk of the biceps, approximately two thirds of the way distally down the arm between the acromion process of the shoulder and the olecranon of the elbow. Participants were instructed to lay their arms as flat as possible on a table so that their arms were parallel to the table with their triceps resting.

All landmarks were mapped using a clear overhead projector sheet to ensure that the sites were measured at the same location during the post-test measurement. A water-based gel was applied to the 5MHz scanning transducer head to allow for optimal sound wave transmission. The transducer was held perpendicular to the skin while avoiding compression of the skin and the underlying tissue. An image of the fat/muscle and muscle/bone interface was frozen on the display screen for measurement. For each site, measurements were taken at three different points of the site: the proximal, the mid, and the distal, as determined by divisions (1 cm) on the monitor. The distal and proximal sites were 6 cm apart, with the mid site located 3 cm between them. Muscle thickness was measured from the fat/muscle interface to the muscle/bone interface. Measurements were taken to the nearest 0.1 mm. Three measurements were taken at each of the three points on each site. An average of these three measurements was taken as the final value. The reliability of these ultrasound measurements ranged from a coefficient of variation of 1 to 3% (Candow and Chilibeck 2005).

2.3.7 Upper respiratory tract infections

At baseline, the participants were given diaries to track any upper respiratory tract infections, flu like symptoms, colds and allergies. The participants were also asked to report the

severity (mild, moderate, severe) and the duration of any of the symptoms. The diaries were collected and analyzed after 8 weeks.

2.4 Statistical Analysis

Body composition values, 1RM results, jumps heights, predicted VO_2max, salivary markers of immune function and inflammation, muscle thickness, dietary variables, training volume, baseline characteristics, and average duration of any respiratory tract infections were assessed by a group (i.e. BC vs. soy protein) by time (baseline vs. 8 weeks) ANOVA with repeated measures on the time factor. If a significant change was found, we used a paired *t*-test for a post-hoc analysis. An ANCOVA was used to assess any outcomes that differed between groups at baseline (i.e. baseline scores were used as the co-variate). Statistical significance accepted at alpha level ≤ 0.05. All data analyses were performed with IBM SPSS Statistics, version 22.0 for Windows.

3. Results:

At baseline, twenty-nine (N=29) participants were randomized (see participants' characteristics- Table 3-1). 5 participants (4 from the BC group, and 1 from the soy group) were lost to follow up, and 2 participants (both from the soy group) withdrew due to gastro-intestinal distress. Hence, the final per-protocol cohort analysis (Figure 3-1; Table 3-2) included twenty-two players participants in total (N=22); 11 participants from the BC group, and 11 participants from the soy group. At baseline, the two groups were similar (Table 3-1), and there were not any statistically significant differences between participants who completed the intervention and those who did not complete the intervention (Table 3-3). Means and SD for all dependent variables, before and after 8 weeks of supplementation, can be found on Table 3-2.

Table 3-1. Participants' baseline characteristics (mean ±SD) before 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season.

Group	**BC (n=15)**	**Soy (n=14)**
# of Females	n=2	n=1
Age (y)	25.7 ±7.0	29.6 ±9.4
Height (cm)	181.1 ±7.8	179.2 ±8.8
Weight (kg)	95.6 ±14.2	89.7 ±17.1
Total lean tissue mass (kg)	67.2 ±9.5	64.5 ±7.9
Legs lean tissue mass (kg)	21.8 ±3.3	21.2 ±2.6
Fat mass (kg)	26.2 ±11.2	23.1 ±12.6
Body fat %	26.2 ±8.7	24.1 ±9.0
Arm thickness (cm)	4.0 ±0.6	3.8 ±0.5
Thigh thickness (cm)	4.5 ±0.8	4.0 ±0.8
1RM bench press (kg)	145.0 ±40.9	131.9 ±26.0
1RM leg press (kg)	274.0 ±56.8	271.7 ±79.6
Predicted VO_2max (mL/kg/min)	40.9 ±9.2	43.3 ±10.5
Vertical jump height (cm)	43.0 ±9.3	44.8 ±8.5

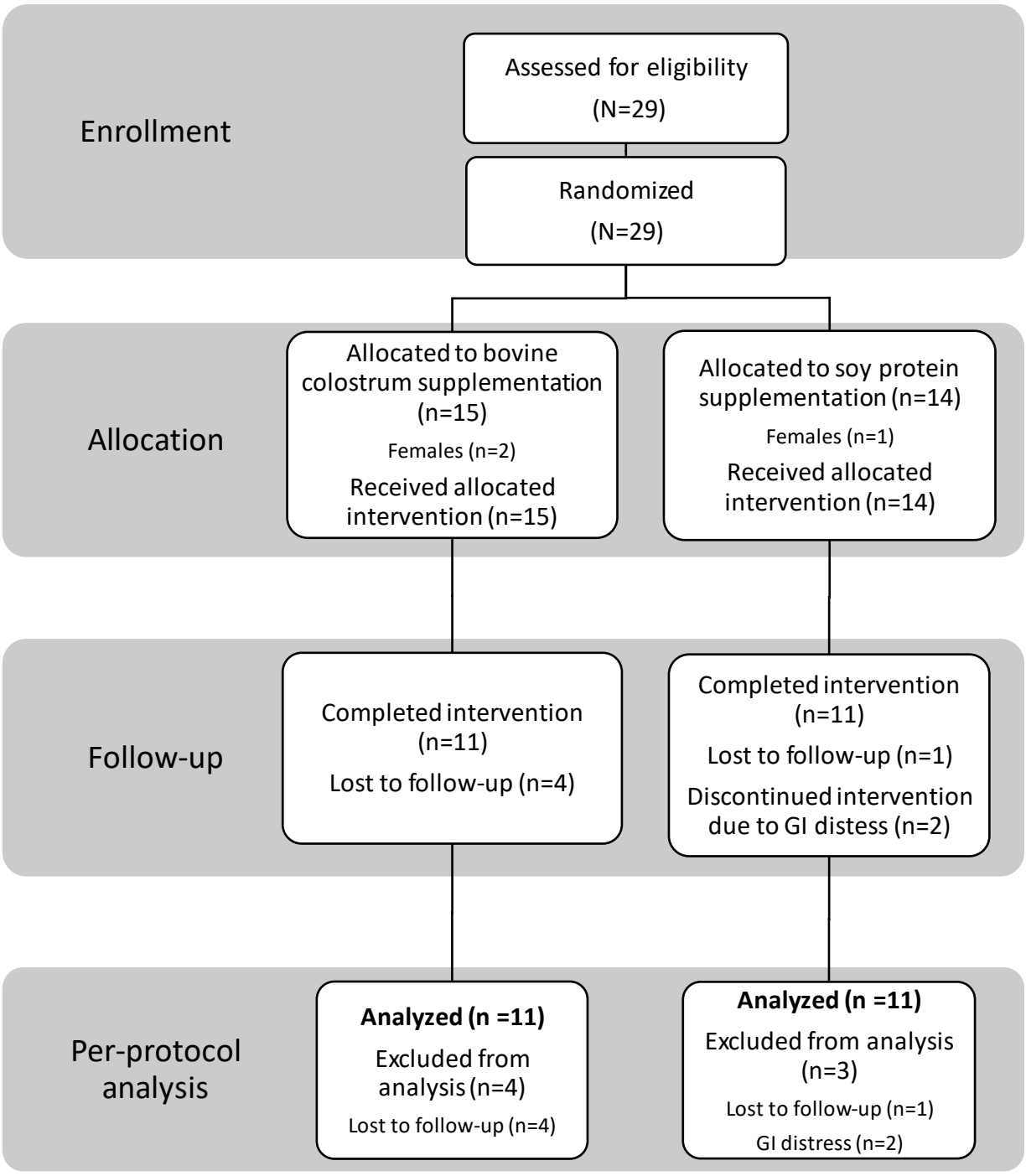

Figure 3-1. Participants flow; per-protocol analysis

Table 3-2. Means and SD for all dependent variables, before and after, 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season.

Dependent Variable	**Time**	**Group**	
		BC (n=11)	**Soy (n=11)**
Weight (kg)	*Pre*	95.6 ± 14.2	90.7 ± 15.2
	Post	94.7 ± 12.7	89.9 ± 15.7
Total Lean Tissue Mass (kg)	*Pre*	66.7 ± 8.7	65.8 ± 7.1
	Post	66.1 ± 8.1	65.2 ± 7.3
Legs Lean Tissue Mass (kg)	*Pre*	21.8 ± 3.3	21.5 ± 2.2
	Post	21.7 ± 3.3	21.6 ± 2.4
Total Fat Mass (kg)	*Pre*	25.6 ± 12.0	21.5 ± 11.7
	Post	25.2 ± 10.8	21.3 ± 11.8
Body Fat (%)	*Pre*	26.0 ± 10.0	22.6 ± 8.3
	Post	26.0 ± 9.1	22.6 ± 8.5
Mean Arm Thickness (cm)	*Pre*	3.9 ± 0.5	3.9 ± 0.4
	Post	4.1 ± 0.4	4.1 ± 0.3
Mean Thigh Thickness (cm)	*Pre*	4.6 ± 0.8	3.9 ± 0.7
	Post	4.7 ± 0.7	4.2 ± 1.2
1RM Bench Press (kg)	*Pre*	144.2 ± 45.2	139.7 ± 20.8
	Post	145.8 ± 48.4	145.9 ± 24.0
1RM Leg Press (kg)	*Pre*	274.8 ± 65.4	281.6 ± 65.4
	Post	270.5 ± 55.3	268.4 ± 55.3
Relative VO_2max (mL/kg/min)	*Pre*	40.6 ± 10.0	43.2 ± 10.6
	Post	43.8 ± 10.8	43.8 ± 10.7
Vertical Jump Height (cm)	*Pre*	42.4 ± 10.1	46.5 ± 8.9
	Post	43.4 ± 9.0	46.0 ± 9.0
IgA (μg/mL)	*Pre*	16.7 ± 282.1	18.3 ± 191.9
	Post	17.1 ± 117.0	18.1 ± 145.6
IL-1β (pg/mL)	*Pre*	230.6 ± 142.4	544.6 ± 810.1
	Post	158.8 ± 128.7	430.0 ± 460.4
CRP (pg/mL)	*Pre*	5034.3 ± 3730	4729.9 ± 5830.7
	Post	1654.4 ± 600.3	3835.0 ± 3864.4
IL-6 (pg/mL)	*Pre*	0.7 ± 0.6	1.6 ± 2.4
	Post	0.3 ± 0.5	1.5 ± 2.8

Table 3-3. Baseline Means and SD for all dependent variables for participants who dropped out and participants who completed the intervention.

Group	BC				Soy			Mean ± SD for drop-outs	Mean ± SD for participants who completed the intervention
Reason for drop-out	lost to follow up (n=4)				lost to follow up (n=1)	GI distress (n=2)			
Age (y)	23	18	21	20	24	28	35	24.1 ± 5.8	28.7 ± 8.8
Height (cm)	187.8	189.0	185.0	176.0	167.0	171.0	180.0	179.4 ± 8.5	180.5 ± 8.3
Total Lean Tissue Mass (kg)	80.2	74.2	75.7	53.2	65.0	47.5	67.0	66.1 ± 12.1	66.3 ± 7.8
Total Fat Mass (kg)	39.3	25.4	30.8	16.0	40.6	10.1	37.1	28.5 ± 11.9	23.5 ± 11.8
Body Fat (%)	31.8	24.6	28.0	22.3	15.0	17.0	35.0	24.8 ± 7.4	24.3 ± 9.1
Mean Arm Thickness (cm)	5.1	4.1	4.8	3.3	3.9	3.8	3.8	4.1 ± 0.6	3.9 ± 0.4
Mean Thigh Thickness (cm)	4.1	4.7	3.5	3.8	5.3	4.0	3.9	4.2 ± 0.6	4.3 ± 0.8
1RM Bench Press (kg)	145.5	163.6	186.4	113.6	109.1	118.0	134.0	138.6 ± 28.6	141.9 ± 34.4
1RM Leg Press (kg)	250.0	286.4	304.5	245.5	327.3	218.0	268.0	271.4 ± 37.5	278.2 ± 70.4
Relative VO_2max (mL/kg/min)	31.5	49.0	38.5	45.5	35.0	38.5	44.0	40.3 ± 6.2	42.0 ± 10.1
Vertical Jump Height (cm)	41.3	50.0	55.0	40.0	35.0	33.0	45.0	42.8 ± 7.9	43.8 ± 9.4

3.1 Body composition

There was no significant interaction between time and type of supplementation for total lean body mass [$F_{time\ x\ group}$ (1,20) = 0.002, p=0.964], arms lean body mass [$F_{time\ x\ group}$ (1,20) = 0.070, p=0.935], trunk lean body mass [$F_{time\ x\ group}$ (1,20) = 0.419, p=0.525], legs lean body mass [$F_{time\ x\ group}$ (1,20) = 0.595, p=0.450], total fat mass [$F_{time\ x\ group}$ (1,20) = 0.138, p=0.715] and body fat percentage [$F_{time\ x\ group}$ (1,20) = 0.008, p=0.929]. Players who consumed BC did not change differently across time in comparison to players who consumed soy protein.

There was a significant main effect of time for total lean body mass which decreased over time [F_{time} (1,20) = 11.754, p=0.003], and also a significant main effect of time for trunk lean body mass which decreased over time [F_{time} (1,20) = 5.349, p=0.031].

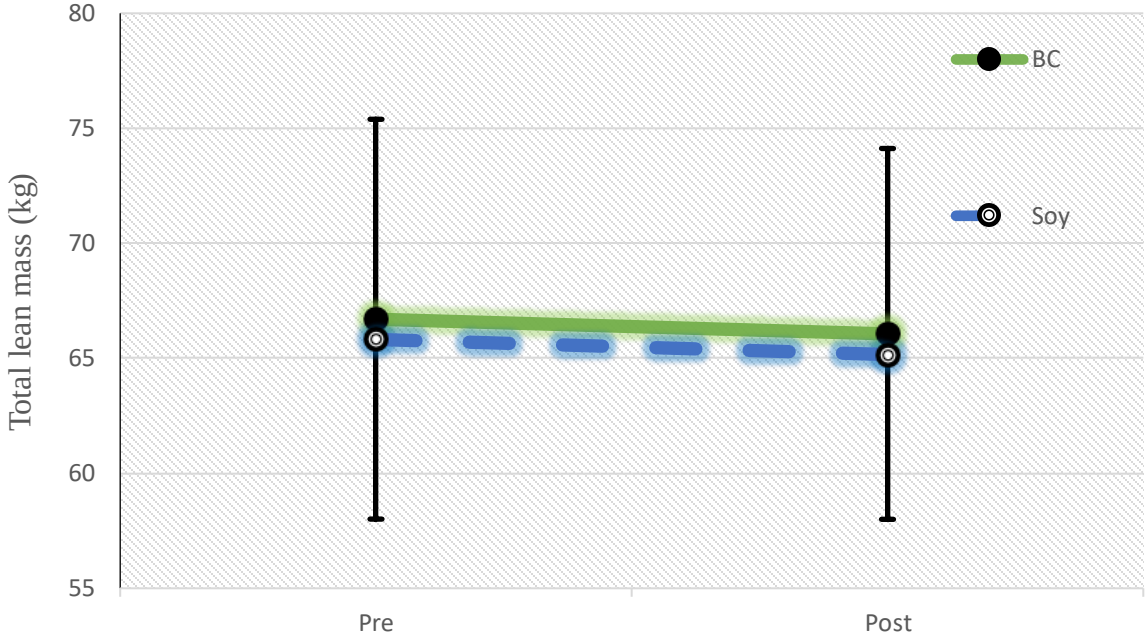

Figure 3-2. Total lean mass (kg) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

3.2 Muscle Thickness

There was no significant interaction between time and type of supplementation for elbow flexors muscle thickness [$F_{time\ x\ group}$ (1,20)= 0.206, p=0.655] and knee extensors muscle thickness [$F_{time\ x\ group}$ (1,20)= 0.842, p=0.370].

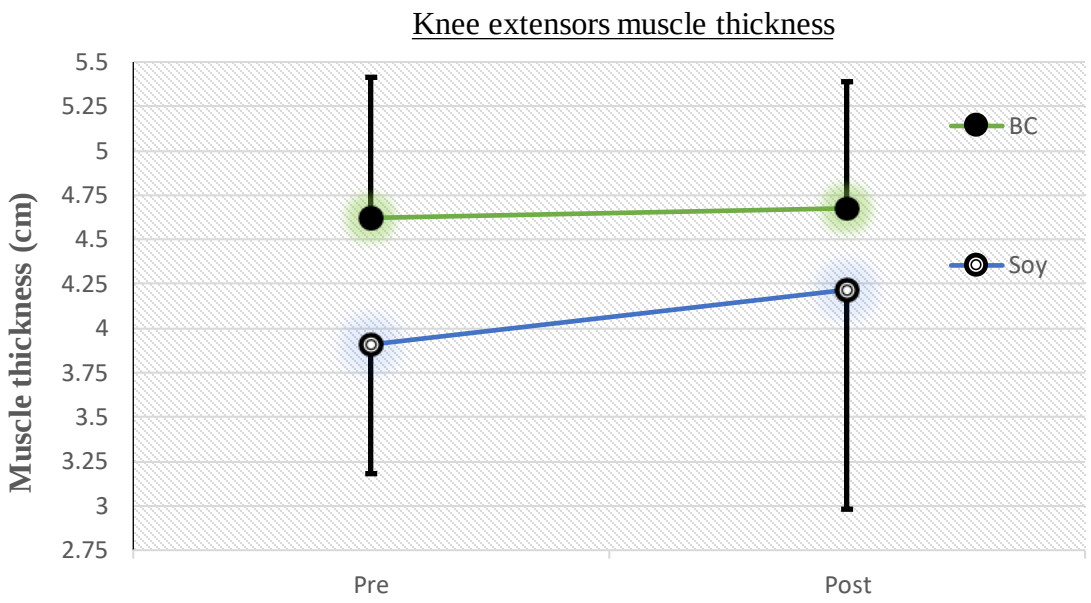

Figure 3-3. Knee extensors muscle thickness (cm) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

There was a significant main effect of time for elbow flexors muscle thickness [$F_{time}(1,20)= 14.434$, $p=0.001$], which increased over time across participants.

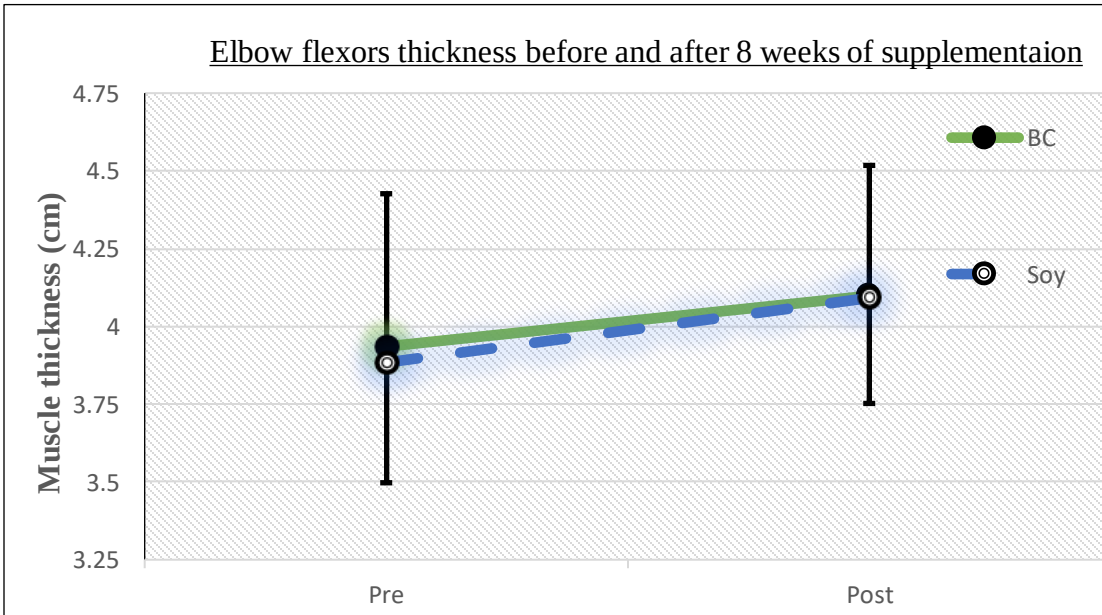

Figure 3-4. Elbow flexors muscle thickness (cm) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

3.3 Strength

There was no significant interaction between time and type of supplementation for bench press 1RM [$F_{time\ x\ group}$ (1,20) = 0.674, p=0.421] and leg press 1RM [$F_{time\ x\ group}$ (1,20) = 0.36, p=0.556]. Players who consumed BC did not change differently across time in comparison to players who consumed soy protein. Also, there was no time main effect for strength.

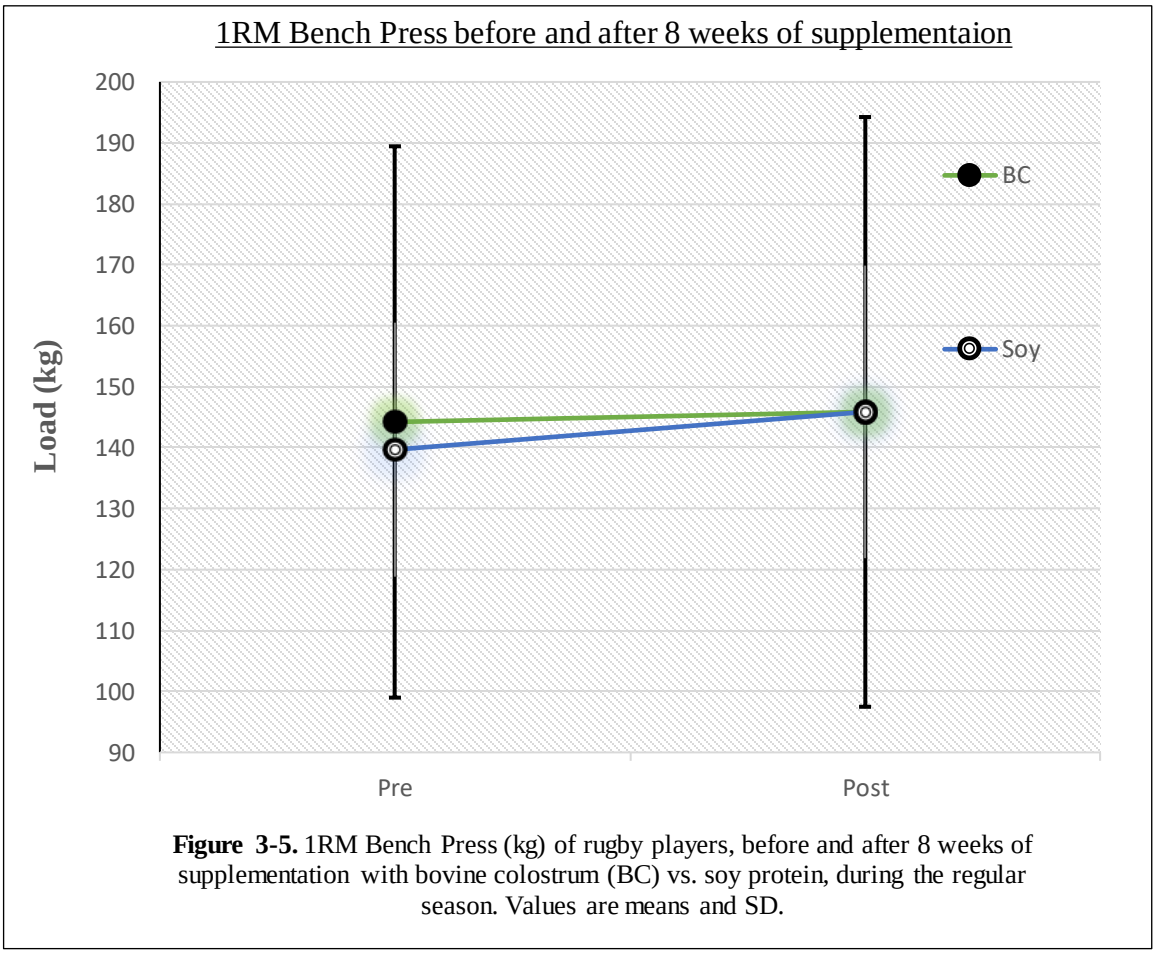

Figure 3-5. 1RM Bench Press (kg) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

The relatively high SDs in both strength exercises are probably due to the difference between player's position on the field (i.e., different physiological demands), and their body size and weight.

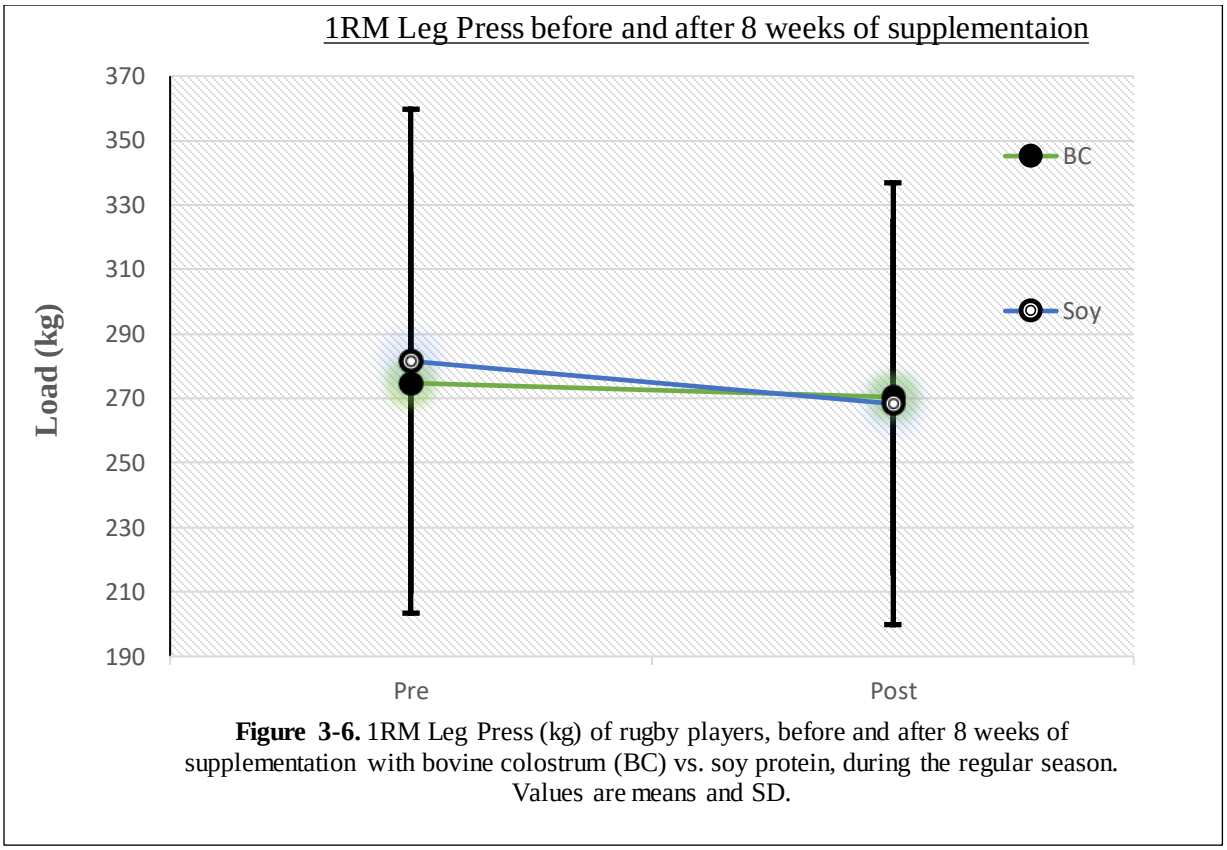

Figure 3-6. 1RM Leg Press (kg) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

3.4 Vertical Jump Height

The analysis included twenty participants (N=20), after two participants (one from each group) could not complete the post testing for vertical jump due to an injury (could not perform shock absorbing movements, such as landing, due to pain).

ANOVA: There was a significant interaction between time and type of supplementation for vertical jump height [$F_{time\ x\ group}(1) = 10.58$, $p = 0.004$]. Players who consumed BC improved their vertical jump over time (+2.5%), in comparison to players who consumed soy protein (Table 3-2). In a post-hoc analysis, using a paired *t*-test we found that pre to post scores for the BC group were significantly different [$F(9) = 4.243$, $p < 0.05$], whereas there was no difference for pre to post scores for the soy group [$F(9) < 0.0001$, $p > 0.05$]. Players who consumed BC improved their vertical jump over time, in comparison to players who consumed soy protein, with an effect size of 0.37.

ANCOVA: To control for baseline differences in vertical jump height, ANCOVA was conducted using baseline values as covariates. The analysis revealed that BC supplementation improved vertical jump height (adjusted post-intervention mean of 45.3 cm) to a greater extent than soy protein supplementation (adjusted post-intervention mean of 42.8 cm) [F (1,17) =8.055, p=0.011].

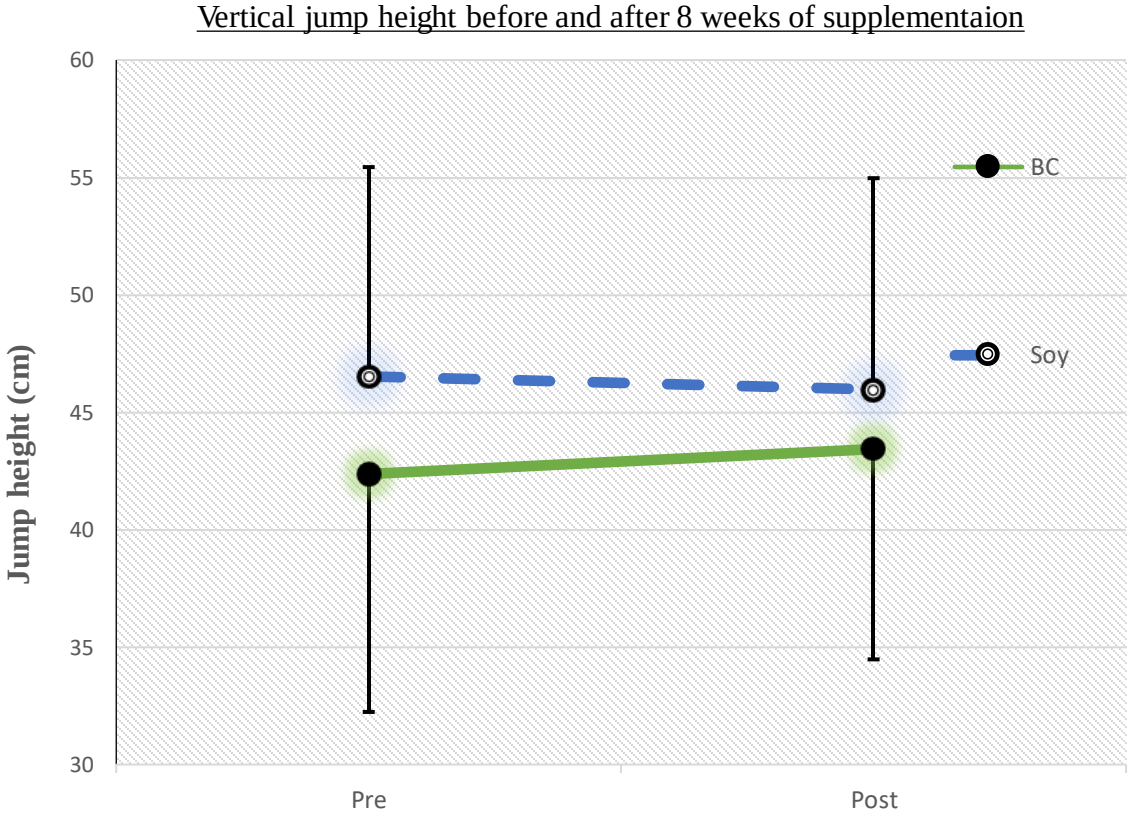

Figure 3-7. Vertical_jump height (cm) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

*ANOVA p< 0.01; **Post hoc t-test p<0.05 ; ***ANCOVA p=0.01

3.5 Aerobic Fitness

One participant (from the BC group) could not finish the post testing for the Leger maximal multistage 20m shuttle run field test (due to an injury), and therefore was not included in the analysis (N=21). There was no interaction between time and type of supplementation on predicted VO_2max [$F_{time\ x\ group}$ (1,19)=2.256, p=0.126]. On the other hand, there was a significant main effect of time, where predicted VO_2max increased over time [F_{time} (1,19) = 5.796, p=0.026].

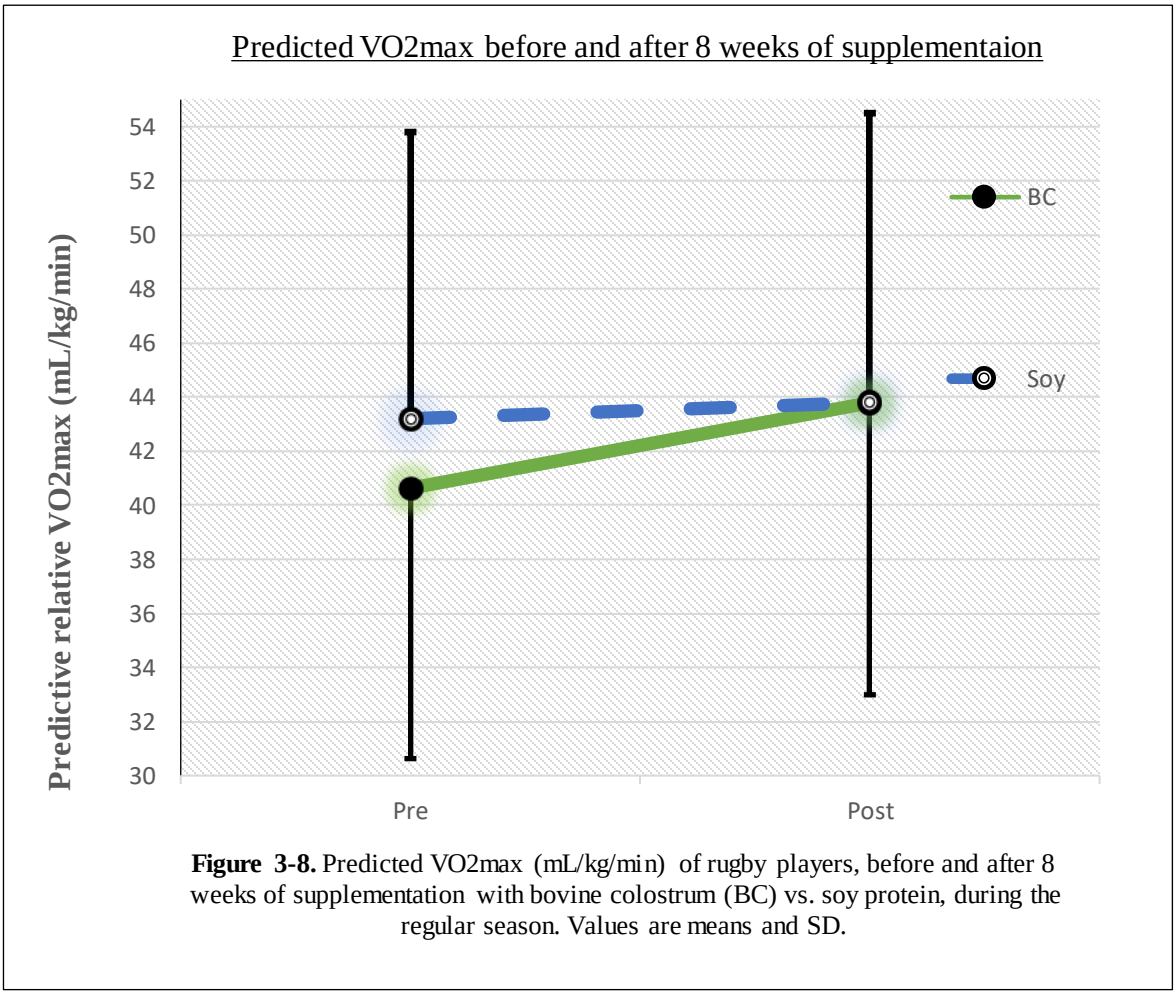

Figure 3-8. Predicted VO2max (mL/kg/min) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

3.6 Markers of immune function

There was no significant interaction between time and type of supplementation for IgA [$F_{time\ x\ group}$ (1,20) = 1.181, p=0.29], IL-1β [$F_{time\ x\ group}$ (1,20) = 0.038, p=0.848], CRP [$F_{time\ x\ group}$ (1,20) = 2.402, p= 0.137], or IL-6 levels [$F_{time\ x\ group}$ (1,20) = 0.037, p=0.848].

There was a significant main effect of time for CRP levels [F_{time} (1,20) = 7.108, p=0.015], with CRP decreasing over time across all participants.

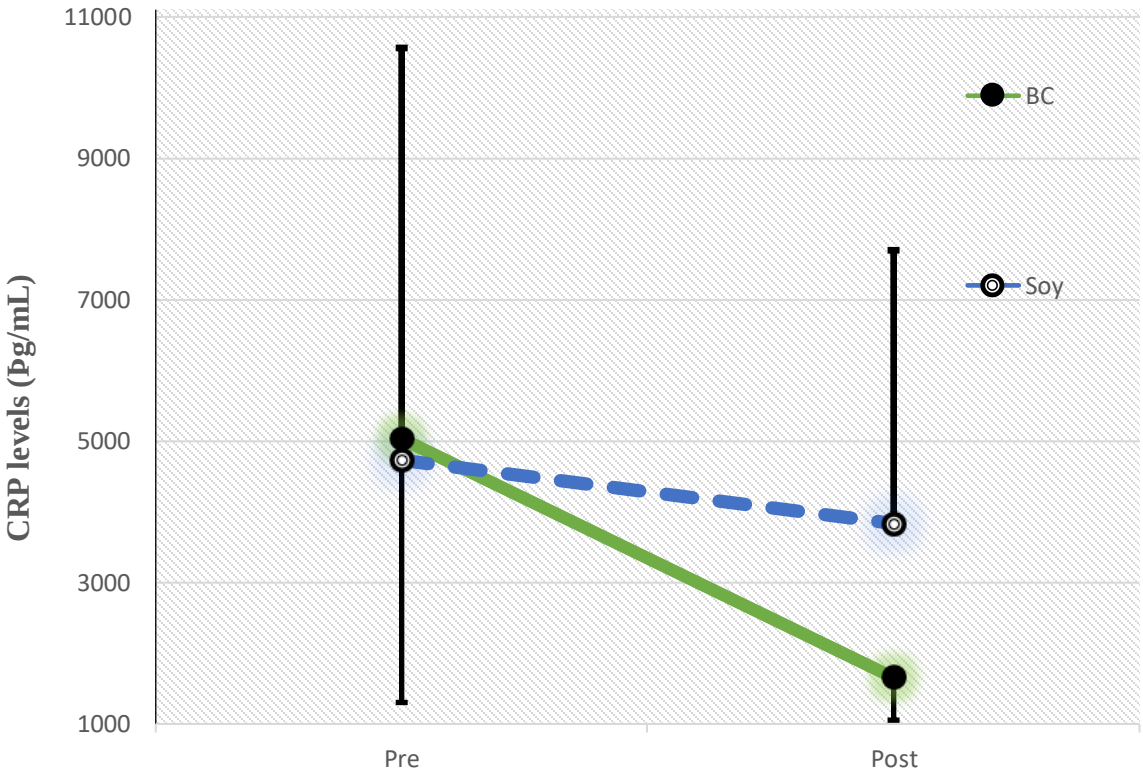

Figure 3-9 .CRP levels (pg/mL) of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. Values are means and SD.

Due to a violation of the Levine test for homogeneity of variance (both before and after the removal of outliers), a non-parametric Mann-Whitney U-test, was used to verify the effect. No

significant difference was found when comparing change scores between the supplement groups (U=48.0; p=0.43).

3.7 Upper respiratory tract infections

No upper respiratory tract infection symptoms were reported throughout the study in either group. Only one participant from the BC group reported that in total, he had 3 days with some allergy symptoms (irritated eyes, runny nose, sneezing). The same participant also reported that he did not have any upper respiratory tract infections symptoms.

3.8 Energy intake

Two participants did not report their energy intake. Hence, only 20 participants were analyzed. There were no differences between groups over time for total energy [$F_{time\ x\ group}$ (1,18)=0.0001, p=0.991], protein [$F_{time\ x\ group}$ (1,18) = 1.216, p=0.285], carbohydrate [$F_{time\ x\ group}$ (1,18)=0.62, p=0.441] and fat [$F_{time\ x\ group}$ (1,18) = 0.09, p=0.768] consumption.

Table 3-4. Total energy, protein, carbohydrate and fat consumption of rugby players, before and after 8 weeks of supplementation with bovine colostrum (BC) vs. soy protein, during the regular season. These values do not include the supplement.

	Energy intake (Kcal)		***Protein (g)***		***Carbohydrate (g)***		***Fat (g)***	
	Pre	***Post***	***Pre***	***Post***	***Pre***	***Post***	***Pre***	***Post***
BC group n=10	3274 (±557)	3240 (±522)	166 (± 24) ~20.3% of total calories	171 (±20) ~21.1% of total calories	340 (±68) ~41.6% of total calories	328 (±67) ~40.5% of total calories	139 (±33) ~38.2% of total calories	138 (±27) ~38.3% of total calories
Soy group n=10	3190 (±307)	3157 (±282)	169 (±28) ~21.2% of total calories	168 (±22) ~21.3% of total calories	349 (±40) ~43.8% of total calories	349 (±38) ~44.2% of total calories	124 (±24) ~35% of total calories	121 (±20) ~34.5% of total calories

4. Discussion

The main finding of the current investigation was that BC supplementation in rugby players did not significantly improve body composition, strength, aerobic fitness, or immune markers level in saliva. A secondary finding of this investigation was that rugby players who consumed BC during the season, significantly improved vertical jump height over time, in comparison to players who consumed soy protein. This is the first study to our knowledge, to show increased vertical jump without any change in body composition and/or strength, in rugby players. The vertical jump finding partially supports our hypothesis that the BC group would have greater increases in some fitness parameters. The hypothesis for improvement in estimated power was based on the evidence that: 12 days of BC supplementation significantly improved jump flight times in comparison to placebo supplementation, in athletes (Leppäluoto et al. 2000); and that BC supplementation significantly improved peak cycle power and vertical jump height, after 8 weeks of resistance and plyometric training (Buckley et al. 2003).

Successful performance during rugby requires high levels of leg power (which can be estimated from vertical jump performance) (Young et al. 1995), particularly for the forwards in scrums, rucks and mauls, where the goal is to keep the other team away from the ball (Miller et al. 2000; Robinson 2000). The mean pack force during scrummaging ranges from 6210–9090N/ ~600–1000kg (Quarrie and Wilson, 2000). Vertical jump or leg power per se, would be important during lineouts (restarting play after the ball has gone into touch) and kick receiving, when players need to jump for the ball. Leg power in general would be important during scrummaging, rucking, and mauling when players are pushing against each other.

4.1 Body Composition

In contrast to our original hypothesis, there were no significant changes for either group in lean tissue mass, body fat percentage (assessed by DXA) and muscle thickness (assessed by ultrasound).

The absence of significant increases in overall lean body mass is not consistent with one study by Antonio et al. (2001), where body mass significantly increased for their whey group, but primarily due to an increase in fat mass, while the BC group showed a significant increase in lean body mass only, without any significant change in bodyweight. Antonio et al. (2001) used a low dosage of only 20g/day, on a non-athlete population, in which body composition changes are more likely, in comparison to athletes or the elderly. However, this finding is consistent with a previous study in our lab, in which Duff et al. (2014) did not find any significant differences in body composition between their two groups of interventions (BC vs. whey supplementation in older adults). In addition, Brinkworth and Buckley (2004) also did not find any significant differences between groups for upper limb muscle cross-sectional area, after similar supplementation and a training regime for the upper limb muscles. Both of the mentioned studies used the dosage of 60g/day and were conducted in populations that are less likely to change their body composition in a drastic way during 8 weeks only (elderly and pro athletes, respectively).

The findings of a positive time main effect (increased muscle mass) for elbow flexors, together with a negative time effect for total lean body (muscle) mass, may seem to contradict each other. However, when analyzing the lean body mass results by region (DXA scans), we found that a significant change (p=0.03) was present in the trunk region only. This means that players lost most of the total lean body mass from their trunk region, while potentially gaining a smaller amount of lean mass in their elbow flexors.

The negative time main effect for lean body mass, and the positive time main effect on aerobic fitness (i.e. decrease in lean tissue mass, but increase in aerobic fitness during our intervention), in addition to similar findings by Gabbett (2005) and Schneider et al. (1998), may suggest that during in-season rugby training programs, teams tend to put an emphasis on skills (catching, ball-carrying, tackling, ball control, etc.), speed, agility, and aerobic training, while neglecting strength training. This might be due to the time constraints (Gabbett 2002).

This hypothesis should be examined in future research, as the negligence of strength training can increase the loss of lean tissue mass and strength, and increase the risk for injuries (Gabbett 2005).

4.2 Strength

In contrast to our original hypothesis, there was not a significant change for either group in strength, measured using the 1RM bench press and 1RM leg press. This finding is supported by Antonio et al. (2001) who supplemented with 20g/day BC for 8 weeks as well; however, it contradicts the finding of a significant difference of strength found by Kerksick et al. (2001), who used a dosage of 60g/day of BC in combination with creatine for 12 weeks. These differences in the supplement content and the duration of supplementation might be the reason for the different result.

In addition, our results also contradict the results by Duff et al (2014), who found a difference in lower body strength only (leg press 1RM), and no difference in upper body strength (bench press 1RM) with BC supplementation. In this case, the different dosage of BC (60g/day) and the different population (elderly), might be the reason for the inconsistency in the results.

4.3 Vertical Jump Height

The significant positive change in vertical jump height, in the BC group, fits our initial hypothesis that BC supplementation would improve vertical jump height to a better extent, in comparison to soy protein supplementation in rugby players. This significant change in the BC group, which may suggest a significant change in lower body power, is the only statistically significant finding in our study and it supports previous evidence found by Leppäluoto et al. (2000), Hofman et al. (2002), and Buckley et al. (2003).

It is important to mention that while we found a significant change of 1.1 cm (2.1%) on average, the standard error of measurement, using the Vertec device, was found to be 2.1 cm (Nuzzo et al. 2011). Hence, this change might be caused by an error of the measurement, may not represent a true change, and might be a type 1 error (rejecting the null hypothesis, when it should not be rejected). On the other hand, while considering the fact that the participants were

amateur athletes, the change magnitude (2.5% in 8 weeks) seems reasonable as Sheppard et al. (2009) found a change of only 1.3% in elite volleyball athletes after 12 months of training, and Markovic et al. (2007) found a change of 6% in non-athletes after 10 weeks of power training.

While this statistically significant change in vertical jump (power) was found without a significant change in strength or lean tissue mass, we can conclude (assuming that the change represents a true change and is not a type 1 error) that:

i: The aspect of power that was affected by the BC supplementation was velocity (i.e. neuromuscular aspects), as power is the product of force (strength) multiplied by velocity. While strength is affected by the nervous and the musculoskeletal systems, velocity is determined by the nervous system; more specifically, by the functioning of the motor cortex (Tyc and Boyajian 2011), cerebellum (Spraker et al. 2012), coordination (Almasbakk and Hoff 1996), and other neuromuscular properties such as the rate of force development (McLellan et al. 2012). Although neurological changes seem like the possible mechanism, we cannot determine whether these changes accrued in the central nervous system or the peripheral nervous system. More research is needed in order to decipher the effect of BC supplementation on these neuromuscular aspects and power.

Or that:

ii: The decrease in total body mass (lean mass + fat mass), while maintaining the same level of strength (Table 3-2), could lead to a higher vertical jump height, due to a lower gravitational (downward) force that is being applied on the participants (lower body weight), resulting in a higher net force upward.

4.4 Aerobic Fitness

We did not find a significant interaction between the groups of supplementation over time. Previous studies found that BC supplementation improves endurance performance. For example, Shing et al. (2006) used the same period of time of supplementation in cyclists, and found that in a treadmill test to exhaustion, the BC group covered a significantly greater distance and completed more work than the whey group and that parasympathetic indices of heart rate variability (i.e., increased intervals between two consecutive R waves in the ECG) were elevated in the BC group and reduced in the whey group, indicating better cardiovascular functioning

with lower heart rate and higher cardiac output in the BC group (2013). Coombes et al. (2002) also found that time-trial performance significantly improved in cyclists who were supplemented with BC when compared with the whey.

The mechanism for improvement in endurance performance is unclear and additional research is needed in order to determine whether BC supplementation improves endurance through the enhancement of nutrient uptake from the intestine, increased muscle glycogen, prevention of decreased testosterone, or a different reason.

4.5 Immune Function

In contrast to our secondary hypothesis, there were no significant interactions between time and type of supplementation for any of the immune function markers.

To our knowledge, this is the first study to measure the effect of BC supplementation on CRP concentration in athletes. More research is needed in order to determine whether BC supplementation decreases CRP concentration, due to the fact that lower CRP levels in athletes can be a beneficial for a faster recovery, better immune functioning and inflammation reduction.

However, from previous studies one can speculate that BC may influence the immune system through the stimulation of cytokine production, as it was shown to:

i) Increase cytokine messenger RNA, therefore increased potential for expression of beneficial cytokines, in cells of intestinal Peyer's patches (lymphoid nodules that form an important part of the immune system by monitoring intestinal bacteria populations and preventing the growth of pathogenic bacteria in the intestines) in weaned piglets (Boudry et al. 2007); and

ii) Stimulate cytokine secretion from peripheral blood mononuclear cells under resting and inflammatory conditions: BC increased the secretion of IFN-γ and IL-10 (anti-inflammatory), in resting conditions, and reduced the secretion of TNF and IL-4 (pro-inflammatory cytokines) and IL-6 (associated with muscle damage), in a culture rich with lipopolysaccharide (Shing et al. 2007). These finding suggest that BC may reduce pro-inflammatory cytokine production following strenuous exercise, which is associated with elevated lipopolysaccharide concentrations. Increased circulating lipopolysaccharide levels in humans, lead to various symptoms, such as fever, shivering, dizziness, nausea, various gastro-intestinal complaints such as vomiting and diarrhoea, and ultimately sepsis (Jeukendrup et al. 2000).

Lastly, based on the literature and the possible increase in salivary IgA concentration, we also hypothesized that BC supplementation would reduce respiratory tract infections. But, as we did not have any cases of respiratory tract infection symptoms, we could not perform an analysis. The reason for the lack of upper respiratory tract infections might be due the winter seasonality of more than 200 different viruses (e.g: influenza, paramyxoviruses, parainfluenza, coronaviruses, picornaviruses) that cause upper respiratory tract infections (Collier and Oxford, 2000), and the fact that our intervention was done during the summer months.

4.6 Strengths, Limitations and Future Research

Other than being the first study, to our knowledge, to examine the effect of BC supplementation in rugby players, this study had a strong design (i.e. double blind, active comparator, and randomized). Moreover, our study had multiple outcomes while presenting a small amount of risks and side effects (only 2 cases were reported in total; both in the soy group, and led the participants to withdraw). Our outcomes were measured in valid, reliable and non-invasive methods such as the DXA scan, that has a coefficient of variation for lean tissue mass of 0.5% (Chilibeck et al. 2013), and the B-mode ultrasound which is a sensitive method to measure muscle thickness and had been validated in comparison to MRI, for the assessment the knee extensors (Miyatani et al., 2002) and elbow flexors (Miyatani et al., 2000) - the two muscle groups that were assessed in our current study.

The supplements that were used in the study were flavorless and odorless, with the same color and texture, and caused low amount of side effects. We have used an absolute dose (in contrast to relative dose) as all previous studies have done it, and comparison of the effect would fit better. Also, using a fixed dose is usually prescribed when supplements are sold in store (this might be a limitation, because participants with a smaller body size, were to receive a larger relative dose). These, in addition to the sensible daily dose of the supplement, might have contributed the low amount of side effects.

On the other hand, our study had some limitations. One of them is the use of soy protein as the control. As whey is more “similar” to BC, and has a better amino acid profile, it would probably be a better comparator than soy.

In addition, the sex distribution of the participants was not equal, with only 3 females out of a total of 29, making it difficult to determine effects of gender. In future research it would be of benefit to include more women, especially given that rugby is now a men's and women's sport at the Olympics. There will therefore be increased need for research on both men's and women's rugby.

While the advantage of per-protocol analysis is that it better reflects the effects of the intervention (Piaggio et al. 2006), it can lead to bias and poor generalizability. Therefore, another limitation in our study is increased chance for a type 1 error, associated with measuring and analysing multiple outcomes, and the use of per-protocol analysis, which excludes participants who did not complete the protocol (i.e., if the reason for the "lost to follow-up" participants was side effects of the supplement, we might erroneously conclude that the supplement was effective because the remaining participants might have tolerated the supplement and improved their different dependent variables). We were not able to obtain measurements from the 2 participants who dropped out of the study (due to gastro-intestinal distress) as they were not able to arrive to our lab on any of the days that we have suggested for post-interception measurements. The advantage of per-protocol analysis is that it better reflects the effects of the intervention (Piaggio et al. 2006), but it can lead to bias and poor generalizability.

Although we did find a significant difference in vertical jump height, we cannot determine the mechanisms by which BC supplementation affects power, as we did not use any neurological tests and made no assessment of muscle quality. Future research could use neurological tests that would test variables such as peak power, average power, rate of force development, coordination, EMG, tests of motor unit recruitment (e.g: interpolated twitch technique), etc. The power assessment should also include upper body power, using a movement such as explosive push-up on a force plate, in order to test if the improvement in power is in the lower body only, or has an all-around effect. In addition, future training programs of the participants should focus on the main dependant variable that is being measure. i.e., while assessing power, a training program that focuses on the enhancement of strength and power, would be more suitable; and while assessing aerobic fitness, a training program that focuses on the enhancement of aerobic training, would be more suitable. Also, using a cross-over design could be used to measure the difference within the athletes, instead of between the randomized groups, decreasing amount of

variability in analyses. This design would take longer and need a washout period in between the two types of supplementation in order to prevent a carry-over effect.

While saliva sampling is a quick, non-invasive, "friendly" process, it may have some disadvantages (in comparison to human serum). For instance, the levels of IL-6, IL-1β and CRP levels are much lower in saliva than in human serum:

- Mean IL-6 levels in serum is 13.7 pg/mL vs. 1.27 pg/mL in saliva (Arican et al. 2005).
- Mean IL-1β levels in serum is 110 pg/mL vs. 8 pg/mL in saliva (Ozeren et al. 2003).
- Mean CRP levels in serum is $5x10^5$ pg/mL vs. 1300 pg/mL in saliva (Clyne and Olshaker, 1999).

Moreover, the salivary immune markers levels can be impacted by eating, drinking, and other environmental and behavioral conditions. These can affect the reliably and accuracy of the results.

5. Summary and Conclusions

The current research was the first to assess the effect of 8 weeks of BC supplementation on rugby players' fitness and immune function, during the regular rugby season. The study main finding was that BC supplementation did not significantly change body composition, strength, aerobic fitness and levels of salivary immune markers, in rugby players, in comparison to soy protein.

A secondary finding of this work is the significant improvement in vertical jump height, which may suggest improvement in lower body power, in the BC group, in comparison to the control group (soy protein).

More research is needed in order to: i) Determine if BC supplementation significantly improves power in athletes; ii) Find the potential mechanism/s for this effect; and iii) Determine BC supplementation's effect compared to other common protein supplements.

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7. Appendix

APPENDIX A: PAR-Q+

CSEP approved Sept 12 2011 version

PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

Regular physical activity is fun and healthy, and more people should become more physically active every day of the week. Being more physically active is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

SECTION 1 - GENERAL HEALTH

	Please read the 7 questions below carefully and answer each one honestly: check YES or NO.	YES	NO
1.	Has your doctor ever said that you have a heart condition OR high blood pressure?	☐	☐
2.	Do you feel pain in your chest at rest, during your daily activities of living, OR when you do physical activity?	☐	☐
3.	Do you lose balance because of dizziness OR have you lost consciousness in the last 12 months? Please answer NO if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4.	Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)?	☐	☐
5.	Are you currently taking prescribed medications for a chronic medical condition?	☐	☐
6.	Do you have a bone or joint problem that could be made worse by becoming more physically active? Please answer NO if you had a joint problem in the past, but it does not limit your current ability to be physically active. For example, knee, ankle, shoulder or other.	☐	☐
7.	Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

If you answered NO to all of the questions above, you are cleared for physical activity.

Go to Section 3 to sign the form. You do not need to complete Section 2.

- Start becoming much more physically active – start slowly and build up gradually.
- Follow the Canadian Physical Activity Guidelines for your age (www.csep.ca/guidelines).
- You may take part in a health and fitness appraisal.
- If you have any further questions, contact a qualified exercise professional such as a CSEP Certified Exercise Physiologist® (CSEP-CEP) or CSEP Certified Personal Trainer® (CSEP-CPT).
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the questions above, please GO TO SECTION 2.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes – please answer the questions on Section 2 of this document and/or talk to your doctor or qualified exercise professional (CSEP-CEP or CSEP-CPT) before continuing with any physical activity programme.

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SECTION 2 - CHRONIC MEDICAL CONDITIONS

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
1.	Do you have Arthritis, Osteoporosis, or Back Problems?		☐ If yes, answer questions 1a-1c	☐ If no, go to question 2
	1a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	1b.	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	☐	☐
	1c.	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	☐	☐
2.	Do you have Cancer of any kind?		☐ If yes, answer questions 2a-2b	☐ If no, go to question 3
	2a.	Does your cancer diagnosis include any of the following types: lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	☐	☐
	2b.	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	☐	☐
3.	Do you have Heart Disease or Cardiovascular Disease? This includes Coronary Artery Disease, High Blood Pressure, Heart Failure, Diagnosed Abnormality of Heart Rhythm		☐ If yes, answer questions 3a-3e	☐ If no, go to question 4
	3a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	3b.	Do you have an irregular heart beat that requires medical management? (e.g. atrial brillation, premature ventricular contraction)	☐	☐
	3c.	Do you have chronic heart failure?	☐	☐
	3d.	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	☐	☐
	3e.	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	☐	☐
4.	Do you have any Metabolic Conditions? This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes		☐ If yes, answer questions 4a-4c	☐ If no, go to question 5
	4a.	Is your blood sugar often above 13.0 mmol/L? (Answer YES if you are not sure)	☐	☐
	4b.	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, and the sensation in your toes and feet?	☐	☐
	4c.	Do you have other metabolic conditions (such as thyroid disorders, pregnancy-related diabetes, chronic kidney disease, liver problems)?	☐	☐
5.	Do you have any Mental Health Problems or Learning Difficulties? This includes Alzheimer's, Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome)		☐ If yes, answer questions 5a-5b	☐ If no, go to question 6
	5a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	5b.	Do you also have back problems affecting nerves or muscles?	☐	☐

Please read the questions below carefully and answer each one honestly: check YES or NO.			YES	NO
6.	Do you have a Respiratory Disease? This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure		☐ If yes, answer questions 6a-6d	☐ If no, go to question 7
	6a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	6b.	Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy?	☐	☐
	6c.	If asthmatic, do you currently have symptoms of chest tightness, wheezing, laboured breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week?	☐	☐
	6d.	Has your doctor ever said you have high blood pressure in the blood vessels of your lungs?	☐	☐
7.	Do you have a Spinal Cord Injury? This includes Tetraplegia and Paraplegia		☐ If yes, answer questions 7a-7c	☐ If no, go to question 8
	7a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	7b.	Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting?	☐	☐
	7c.	Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia)?	☐	☐
8.	Have you had a Stroke? This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event		☐ If yes, answer questions 8a-c	☐ If no, go to question 9
	8a.	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer NO if you are not currently taking medications or other treatments)	☐	☐
	8b.	Do you have any impairment in walking or mobility?	☐	☐
	8c.	Have you experienced a stroke or impairment in nerves or muscles in the past 6 months?	☐	☐
9.	Do you have any other medical condition not listed above or do you live with two chronic conditions?		☐ If yes, answer questions 9a-c	☐ If no, read the advice on page 4
	9a.	Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months OR have you had a diagnosed concussion within the last 12 months?	☐	☐
	9b.	Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)?	☐	☐
	9c.	Do you currently live with two chronic conditions?	☐	☐

Please proceed to Page 4 for recommendations for your current medical condition and sign this document.

PAR-Q+

If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active:

- It is advised that you consult a qualified exercise professional (e.g., a CSEP-CEP or CSEP-CPT) to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20-60 min. of low- to moderate-intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate-intensity physical activity per week.
- If you are over the age of 45 yrs. and NOT accustomed to regular vigorous physical activity, please consult a qualified exercise professional (CSEP-CEP) before engaging in maximal effort exercise.

If you answered YES to one or more of the follow-up questions about your medical condition:

- You should seek further information from a licensed health care professional before becoming more physically active or engaging in a fitness appraisal and/or visit a or qualified exercise professional (CSEP-CEP) for further information.

Delay becoming more active if:

- You are not feeling well because of a temporary illness such as a cold or fever – wait until you feel better
- You are pregnant - talk to your health care practitioner, your physician, a qualified exercise profesional, and/or complete the PARmed-X for Pregnancy before becoming more physically active OR
- Your health changes - please talk to your doctor or qualified exercise professional (CSEP-CEP) before continuing with any physical activity programme.

SECTION 3 - DECLARATION

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The Canadian Society for Exercise Physiology, the PAR-Q+ Collaboration, and their agents assume no liability for persons who undertake physical activity. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.
- If you are less than the legal age required for consent or require the assent of a care provider, your parent, guardian or care provider must also sign this form.
- Please read and sign the declaration below:

I, the undersigned, have read, understood to my full satisfaction and completed this questionnaire. I acknowledge that this physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if my condition changes. I also acknowledge that a Trustee (such as my employer, community/fitness centre, health care provider, or other designate) may retain a copy of this form for their records. In these instances, the Trustee will be required to adhere to local, national, and international guidelines regarding the storage of personal health information ensuring that they maintain the privacy of the information and do not misuse or wrongfully disclose such information.

NAME _____ DATE _____

SIGNATURE _____ WITNESS _____

SIGNATURE OF PARENT/GUARDIAN/CARE PROVIDER _____

For more information, please contact:
Canadian Society for Exercise Physiology
www.csep.ca

KEY REFERENCES

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The PAR-Q+ was created using the evidence-based AGREE process (1) by the PAR-Q+Collaboration chaired by Dr. Darren E. R. Warburton with Dr. Norman Gledhill, Dr. Veronica Jamnik, and Dr. Donald C. McKenzie (2). Production of this document has been made possible through financial contributions from the Public Health Agency of Canada and the BC Ministry of Health Services. The views expressed herein do not necessarily represent the views of the Public Health Agency of Canada or BC Ministry of Health Services.

CSEP approved Sept 12 2011 version

APPENDIX B: Consent Form

Participant Information and Consent Form

Title: The effect of bovine colostrum and soy protein supplementation on fitness, muscle mass, inflammation and immune function during intense training in rugby players

Principal Investigator: Philip D. Chilibeck, Ph.D., Professor, College of Kinesiology, University of Saskatchewan, phone: 306-966-1072

Student Researcher: Eliran Mizelman, B.Sc., (M.Sc. student supervised by Philip Chilibeck), College of Kinesiology, University of Saskatchewan, phone: 306-966-1123

Sponsors: Mitacs Accelerate Program and the Saskatoon Colostrum Co. Ltd.

Emergency Telephone Number: 306-230-3849

Introduction

You are invited to take part in this research study that involves nutritional supplementation with bovine colostrum or soy protein during your rugby season. Bovine colostrum is the milk that is secreted by cows during the first day after calving. The bovine colostrum and soy protein supplements we are using for this study are commercially available.

Your participation is voluntary. It is up to you to decide whether or not you wish to take part. If you decide to participate, you are still free to withdraw at any time and without giving any reasons for your decision. If you do not wish to participate, you will not affect your relationship with the researchers, the university, or your coaches.

Please take time to read the following information carefully. You can ask the study staff to explain any information that you do not clearly understand. You may ask as many questions as you need. Please feel free to discuss this with your family, friends or family physician before you decide.

Who is conducting the study?

The study is being funded by the Saskatoon Colostrum Co. Ltd. with matching funds from Mitacs, which is a government funding organization that supports research that collaborates with industry. The sponsors of this study will reimburse the investigators and university for the cost of undertaking this study.

Why is this study being done?

This study is being done because we want to determine the effect of soy protein and colostrum protein supplementation during your rugby season and their effects on muscle mass, strength, aerobic performance, and immune function.

Who can participate in this study?

You are eligible to participate in this study if you are male or female training on a regular basis with a rugby club and 18y or older. We will determine whether the exercise testing we will be doing is safe for you by having you fill out a brief questionnaire (the Physical Activity Readiness Questionnaire). If there is doubt about the safety of the exercise testing for you based on this questionnaire, we will need to get permission from your family physician, with your approval, before you can participate in this study. You should not participate in this study if you have allergies to milk or soy.

What does the study involve?

The study involves consuming either the bovine colostrum or soy protein supplement. If you agree to participate in the study you will be randomly assigned (i.e. by chance by a computer) to a group that receives 60 grams per day bovine colostrum supplement or 60 grams per day soy protein. You will have a 50% chance of being assigned to either group. You will be required to consume the protein supplement combined with water, milk, or juice three times per day. On training days, you will consume the supplement before and after your training sessions and then once with a meal. On days when you are not training you will consume the supplement 3 times per day with meals. The study is "double blind" which means that neither you nor the study personnel will know what supplement you are receiving until the end of the study. In case of emergency however, we can find out which supplement you are on.

Before and after the 8 week protein supplementation we will do the following assessments on you:

1) Lean tissue and fat mass will be assessed with dual energy X-ray absorptiometry by a nuclear medicine technologist in the RJD Williams Building at the University of Saskatchewan (221 Cumberland Ave North, Room 108). This involves lying on a table while you are scanned with an X-ray. This test takes about 10 minutes.

2) The size of the muscles at the front and back of your upper arms and legs will be assessed by ultrasound by a Kinesiology research assistant at the RJD Williams Building (221 Cumberland Ave. North, Room 108). This involves placing a gel on your skin and placing a probe over the gel. The probe emits sound waves that allow assessment of the thickness of your muscles. This will take about 20 minutes.

3) Your power and strength will be determined by assessing your vertical jump performance and the maximal amount of weight you can lift during a "bench press" exercise and a "leg press" exercise. The vertical jump test requires you to jump as high as you can. This test will be repeated three times, with your highest jump recorded. The bench press involves lying on a

bench and pushing a weight up from chest level. The leg press involves pushing a weight with your feet while extending your legs. You will initially be given a warm-up on an exercise bike, following by light stretching, and lifting light weights on the bench press and leg press. This power and strength testing will take about 25 minutes. This will be supervised by a Kinesiology research assistant at the RJD Williams Building (221 Cumberland Ave. N., Room 108).

4) We will get you to "drool" into a collection container so we can analyze your saliva for proteins involved in immune function and inflammation. This takes about 5 minutes.

5) You will be required to perform an aerobic exercise test. This involves running between two cones placed 20 m apart at a pace that gradually increases. The pace at which you run will be indicated by "beeps" emitted from a CD player. The test ends when you cannot keep pace with the beeps. This test will last between six and 15 minutes, depending on your aerobic fitness (the test will be longer if you have a higher aerobic fitness). This testing will be done in the RJD Williams Building (221 Cumberland Ave. N, 2nd floor gymnasium).

6) You will be required to keep a record of all the foods and beverages that you consume over a 3 day period. This will be done during the first week and last week of your supplementation. The purpose of this food and beverage diary is so that we can monitor the influence of any foods and beverages you consume on changes in your strength and muscle mass.

7) You will be required to keep a log of your rugby training and games played and any other training you do outside of rugby practices.

8) You will be required to keep a record of any colds you catch during the 8-week study. You will need to indicate the number of colds you catch and the number of days the cold lasts.

All testing (except the food diaries, training logs, and records of any colds) will be done during 2 study visits – one at baseline (i.e. before the supplementation program) and one after the 8-week supplementation program. We anticipate that each of these visits for measurements will last between 75 and 90 minutes.

There will be a total of 36 participants in this study at the University of Saskatchewan.

What are the benefits of participating in this study?

You may increase your muscle mass, strength, and aerobic performance by participating in this study. These benefits are not guaranteed.

What are the possible risks and discomforts?

Bovine colostrum supplementation: In a previous study of older individuals taking the same dose of colostrum, 10% of participants reported mild gastro-intestinal symptoms that included bloating, nausea, and diarrhea. These adverse events were not severe enough for the participants to stop taking the supplement.

The exercise testing may result in muscle pulls or strains, or muscle soreness. You will be given a proper warm-up prior to exercising testing and qualified exercise trainers will supervise testing sessions.

The ultrasound measurements may involve discomfort as the gel used might feel cold.

There is a small amount of radiation exposure from the dual energy X-ray scans. This is equal to one tenth of the amount of radiation you would receive from taking a trans-Atlantic flight from North American to Europe, or less than 0.5% from what you would receive from a routine full-mouth dental X-ray.

What are alternatives to the study?

You do not have to participate in this study to receive the nutritional supplements. The nutritional supplements being evaluated in this study are available at health food stores.

What happens if I decide to withdraw?

Your participation in this research is voluntary. You may withdraw from this study at any time. You do not have to provide a reason. Your relationships with the researchers, the university, or your coaches will not be affected.

If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment will be retained for analysis.

What happens if something goes wrong?

In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Inform the medical staff you are participating in a clinical study. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights against the sponsor, investigators or anyone else.

What happens after completion of the study?

We will inform you of the overall study results after we have analyzed all data (approximately September 2014).

What will the study cost me?

You will not be charged for the study supplements or any research-related procedures. You will not be paid for participating in this study. Reimbursement for study-related expenses (e.g. travel, parking) is not available.

There is a possibility the results of the study might be commercialized to promote the protein supplements. You will not receive any financial benefit from this commercialization.

Will my participation be kept confidential?

In Saskatchewan, the Health Information Protection Act (HIPA) defines how the privacy of your personal health information must be maintained so that your privacy will be respected. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

Who do I contact if I have questions about the study?

If you have any questions or desire further information about this study before or during participation, you can contact Philip Chilibeck at 306-966-1072 or phil.chilibeck@usask.ca

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, contact the Chair of the University of Saskatchewan Research Ethics Board, at 306-*966-2975 (out of town calls 1-888-966-2975)*. The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Research Ethics Board.

CONSENT TO PARTICIPATE

- I have read (or someone has read to me) the information in this consent form.
- I understand the purpose and procedures and the possible risks and benefits of the study.
- I have been informed of the alternatives to the study.
- I was given sufficient time to think about it.
- I had the opportunity to ask questions and have received satisfactory answers.
- I am free to withdraw from this study at any time for any reason and the decision to stop taking part will not affect my future relationships at the university, or with my coaches.
- I agree to follow the principal investigator's instructions and will tell the principal investigator at once if I feel I have had any unexpected or unusual symptoms.
- I have been informed there is no guarantee that this study will provide any benefits to me.
- I give permission for the use and disclosure of my de-identified personal information collected for the research purposes described in this form.
- I understand that by signing this document I do not waive any of my legal rights.
- I will be given a signed and dated copy of this consent form.
- If required, my family physician can be consulted regarding my health and participation in this study.
  - ❑ Yes, you may contact my primary care physician
  - ❑ No, please do not contact my primary care physician
  - ❑ I do not have a primary care physician.

- I agree to participate in this study:

Printed name of participant: _____

Signature _____ Date_____

Printed name of person obtaining consent: _____

Signature _____ Date_____

APPENDIX C: Salivary Immune Markers ALISA Kits

CRP

SALIVARY C-REACTIVE PROTEIN

For Research Use Only

Item No. 1-3302, (Single) 96-Well Kit;

1-3302-5, (5-Pack) 480 Wells

Updated: February 26, 2014

Salimetrics, LLC
101 Innovation Blvd., Suite 302 State College, PA 16803, USA
(T) 814-234-7748, (F) 814-234-1608
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Intended Use

The Salimetrics™ CRP kit is an enzyme-linked immunoassay specifically designed and validated for the quantitative measurement of salivary CRP. It is not intended for diagnostic use. It is intended only for research use.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

C-reactive protein (CRP) is the best-known member of a group of acute-phase proteins, which increase their concentrations during certain inflammatory disorders. CRP is widely used as a bio-marker of inflammation in the body.

Most CRP is produced in the liver, and increased production during the acute phase is induced principally by the cytokine interleukin-6 (IL-6), operating primarily at the level of transcription. (1) IL-6 is released by a variety of tissues, including activated leukocytes, adipocytes, and endothelial cells. (2,3) In turn, CRP is capable of binding to and modulating the function of monocytes, enhancing their capacity to produce inflammatory cytokines, including IL-6. (4,5) CRP binds to phosphocholine, a common constituent of polysaccharide coatings of bacterial pathogens and of cell membranes. This allows it to function as an opsonin, facilitating phagocytosis of pathogens and dead or dying cells. (1,5) Other functions of CRP include activating the classical complement pathway, activating macrophage tumoricidal activity, and protecting against septic shock. (5)

CRP levels in humans are normally quite low, but they increase several hundred fold during the acute-phase response. Elevated serum CRP levels have been associated with the presence of cardiovascular disease. (6,7) Numerous recent research studies investigating serum CRP and its relationship to other diseases have also been carried out. These include hypertension, (8,9) diabetes, (2,10) cancer, (11) and autoimmune disorders. (12) Recent literature suggests possible links between oral health and chronic infection, inflammation, and heart disease. (13) Studies have also linked elevated serum CRP levels to oral contraceptive use.
(14,15)

Recent studies have begun to examine the relationship between salivary and serum CRP. One study reported a moderate to strong association between CRP measured in saliva and in serum, while a second longitudinal study found that salivary and plasma CRP were moderately associated cross-sectionally and across two years. (16,17)

Test Principle

A microtitre plate is coated with mouse antibodies to human CRP. CRP in standards and unknowns and goat anti-human CRP antibodies linked to horseradish peroxidase are added. A "sandwich" is formed with the pre-coated antibody on the bottom, the CRP in the middle, and the antibody linked to horseradish peroxidase on the top. After incubation, unbound components are washed away. Bound CRP peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 2-molar sulfuric acid. Optical density is read on a standard microplate reader at 450 nm. The amount of CRP peroxidase detected is directly proportional to the amount of CRP present. (18)

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Plot the reference standard concentrations on the X axis and the corresponding average optical density on the Y axis.
3. Using the average optical density values of the controls and unknowns, determine the corresponding concentration of CRP in pg/mL from the standard curve. We recommend using a linear curve fit.
4. Multiply the calculated concentrations by the dilution factor of 10 to obtain final CRP concentrations in pg/mL.
5. Samples with CRP values greater than 3000 pg/mL should be diluted with CRP sample diluent and rerun for accurate results. If an addi tional dilution of the sample is used, multiply the results by the dilu tion factor.

Salivary CRP Example Values from Healthy Adults,

Aged 20-54 Years* (16)

N	Mean (pg/mL)	Std Error of Mean (pg/mL)	Range (pg/mL)
51	1293.28	140.61	113.69 - 6131.40

SALIVARY SECRETORY IgA

ENZYME IMMUNOASSAY KIT

For Research Use Only

Item No. 1-1602, (Single) 96-Well Kit;

1-1602-5, (5-Pack) 480 Wells

Updated: February 26, 2014

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Intended Use

The Salimetrics™ SIgA kit is an indirect competitive immunoassay designed and validated for the quantitative measurement of SIgA in saliva samples. It is intended only for research use in humans and some animals.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Secretory immunoglobulin A (SIgA) is the dominant immunoglobulin in external secretions that bathe mucosal surfaces (respiratory, intestinal, and reproductive), where it acts as a key component of the immune system's "first line of defense" against microbial invasion. (1,2) Dimeric IgA secreted by mucosal plasma cells adjacent to the salivary glands is bound and transported through the salivary cells by a polymeric Ig receptor (pIgR). The IgA dimer, in complex with a fragment of the pIgR polypeptide, is then released into saliva as secretory IgA (SIgA). (2,3)

IgA-producing plasma cells are generally undetectable in the mucosae before 10 days of age, but they increase rapidly thereafter. (1) SIgA levels are generally thought to be very low in newborn infants and to rise quickly during the first month of life. (4,5,6) Levels of salivary SIgA continue to increase as children age, stabilizing within the adult range around 5-7 years. (6,7,8) Reports of changes in SIgA secretion in old age are inconsistent, with both increases and decreases having been observed. (9,10)

Relative numbers of IgA-producing plasma cells are higher in the submandibular and sublingual glands compared to the parotid glands, and even higher in certain minor glands, leading to differing levels of SIgA in the secretions from these glands. It is speculated that greater density of plasma cells in certain glands may be due to increased antigenic interactions in those parts of the mouth. (3)

The active transport mechanism into saliva for SIgA serves as a ratelimiting factor, (2) and salivary levels of SIgA decrease as flow rates increase. (7,10) The contributions to whole saliva from the various salivary glands in the mouth also vary greatly according to the rate of flow. (3) The literature therefore recommends that variability in salivary flow rate should be taken into account when estimating salivary levels of SIgA and making comparisons between individuals. (2,3,7)

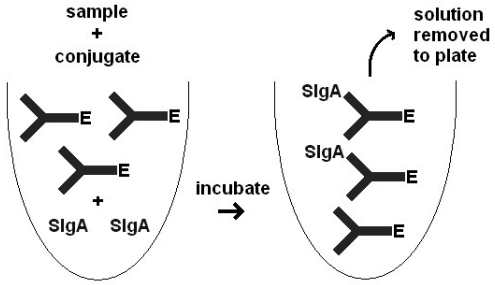
Salivary SIgA levels vary in a complex fashion in response to stress, mood, and emotionality. (2)

The present enzyme immunoassay protocol represents a significant advance over the traditional SIgA measurement approach to employing single radial immunodiffusion (SRID). This enzyme immunoassay

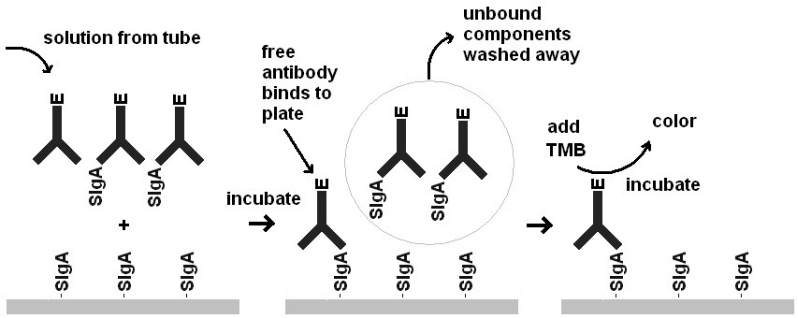
is designed to capture the full range of salivary SIgA levels and uses only 25 µl of saliva per test, with minimal incubation times.

Test Principle

A constant amount of goat anti-human SIgA conjugated to horseradish peroxidase is added to tubes containing specific dilutions of standards or saliva. The antibody-conjugate binds to the SIgA in the standard or saliva samples. The amount of free antibody remaining is inversely proportional to the amount of SIgA present.



After incubation and mixing, an equal solution from each tube is added in duplicate to the microtitre plate coated with human SIgA. The free or unbound antibody-conjugate binds to the SIgA on the plate. After incubation, unbound components are washed away. Bound conjugate is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction. Optical density is read on a standard plate reader at 450 nm. The amount of peroxidase is inversely proportional to the amount of SIgA present in the sample. (11)



Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	B	B/Bo	SIgA (µg/mL)
A1,A2	S1	0.140	0.130	0.079	600.0
B1,B2	S2	0.313	0.303	0.185	200.0
C1,C2	S3	0.526	0.516	0.314	66.7
D1,D2	S4	0.970	0.960	0.585	22.2
E1,E2	S5	1.326	1.316	0.801	7.4
F1,F2	S6	1.509	1.499	0.913	2.5
G1,G2	Bo	1.652	1.642	NA	0
H1,H2	NSB	0.010	NA	NA	NA

Salivary SIgA Example Range*

Group	Number	Range (µg/mL)	Mean (µg/mL)	Std Dev (µg/mL)
Adults	21	93.2-974.03	379.39	261.47

*To be used as a guide only. Each laboratory should establish its own range.

SALIVARY INTERLEUKIN-6 (IL-6)

ELISA KIT

For Research Use Only
Not for use in Diagnostic
Procedures

Item No. 1-3602 (Single) 96-Well
Kit; 1-3602-5 (5-Pack) 480 Wells

SALIVARY IL-6 ELISA KIT

Intended Use

The Salimetrics™ IL-6 kit is a sandwich immunoassay specifically designed for the quantitative measurement of salivary IL-6. It is not intended nor validated for diagnostic use. It is intended only for research use in humans.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine involved in a multitude of inflammatory responses with roles in immune regulation [1,2] and pathologic conditions including both acute and chronic inflammatory diseases [3]. IL-6 initiates and up-regulates inflammation, triggers the release of acute phase proteins, regulates inflammatory response, attracts immune cells to sites of injury or infection and stimulates coagulation [4]. Salivary levels have varying correlations to serum levels depending on the research applications [5, 6].

Test Principle

A microtitre plate is coated with mouse antibodies to IL-6. IL-6 in standards and unknowns attach to the antibody binding sites. After incubation, unbound components are washed away. Biotin conjugated to goat antibodies to human IL-6 are added and attach to the bound IL-6. After incubation, unbound

components are washed away. Streptavidin conjugated to horseradish-peroxidase (HRP) is added and binds to the biotin conjugated to the goat antibodies. Bound streptavidin-HRP is measured by the reaction of the HRP enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 2-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of streptavidin-HRP detected is proportional to the amount of IL-6 present.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	IL-6 (pg/mL)
A1,A2	S1	1.719	100.0
B1,B2	S2	0.888	50.0
C1,C2	S3	0.459	25.0
D1,D2	S4	0.240	12.5
E1,E2	S5	0.133	6.25
F1,F2	S6	0.078	3.125
G1,G2	S7	0.053	1.56
H1,H2	0	0.028	0.0

Limitations

- Samples with IL-6 values greater than 100 pg/mL (or greater than 500 pg/mL after multiplying by the factor of 5 in Step 3) should be diluted further with IL-6 Sample Diluent and rerun for accurate results. Multiply the results by the dilution factor.
- See Specimen Collection recommendations to insure proper collection of saliva specimens and to avoid interfering substances.
- Samples collected with sodium azide are unsuitable for this assay.
- Avoid more than 2 freeze-thaw cycles after the initial freeze/thaw.

Salivary IL-6 Example Ranges*

Nineteen adult subjects of presumably good health collected their own saliva during the day. Samples were frozen within 4 hours. The samples were tested in duplicate with the Salimetrics Salivary IL-6 ELISA Kit.

Salimetrics IL-6 ELISA Kit				
	N	**Median (pg/mL)**	**Min (pg/mL)**	**Max (pg/mL)**
Adult Passive Drool	19	1.27	0	5.37

*To be used as a guide only. Each laboratory should establish its own range.

SALIVARY IL-1β

ELISA KIT

For Research Use Only

Item No. 1-3902, (Single) 96-Well Kit;

1-3902-5, (5-Pack) 480 Wells

Updated: February 26, 2014

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Intended Use

The Salimetrics™ IL-1β kit is a sandwich immunoassay specifically designed for the quantitative measurement of salivary IL-1β. It is not intended nor validated for diagnostic use. It is intended only for research use in humans.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in false values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

IL-1β is a key pro-inflammatory cytokine that is released after infection, injury, or antigenic challenge. It has been widely studied for its role as a multi-functional signaling molecule that affects nearly all cell types, either alone or in combination with other pro-inflammatory cytokines. (1,2)

Circulating IL-1β is principally produced by activated macrophages, but it is also secreted from a wide variety of epidermal, epithelial, lymphoid, and vascular tissues. (1,2) IL-1β is also present in the saliva of both healthy and diseased individuals. In the oral cavity, the sources of IL-1β production include macrophages, monocytes, fibroblasts, and mucosal epithelial cells. (3,4) IL-1β has also been reported to be synthesized

and released from acinar and ductal cells in mouse salivary glands. (57) Human tear fluid and gingival crevicular fluid (GCF), which may be present as components of whole saliva, also contain IL-1β. (8,9)

Salivary IL-1β has been reported to have a circadian rhythm with highest levels in the morning (after waking) and lowest levels in the evening. (10-12) This is the opposite of the pattern seen in serum, where peak levels occur from 1-4 AM and daytime levels are low. (13)

Levels of IL-1β in saliva and GCF have been studied in relation to gingival and periodontal disease, and significant correlation to the presence of periodontal disease has been found. (8,14-19)

Levels of IL-1β in saliva and GCF have been observed to change in response to various types of physical and psychological stressors, similar to the response to seen in the circulation. (10,20-22)

The relationship between salivary and circulating levels of IL-1β is not clear. Serum or plasma levels of IL-1β in healthy individuals are very low and often below the limit of detection. (1,23,24) IL-1β levels are generally higher in saliva than in plasma or serum. (23,25) One study has reported that the correlation between human passive drool saliva samples and plasma for IL-1β is not statistically significant. (26)

Test Principle

A microtitre plate is coated with mouse antibodies to IL-1β. IL-1β in standards and unknowns attach to the antibody binding sites. After incubation, unbound components are washed away. Biotin conjugated to goat antibodies to human IL-1β are added and attach to the bound IL-1β. After incubation, unbound components are washed away.

Streptavidin conjugated to horseradish-peroxidase (HRP) is added and binds to the biotin conjugated to the goat antibodies. Bound streptavidin-HRP is measured by the reaction of the HRP enzyme on the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with 3-molar sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of streptavidin-HRP detected is proportional to the amount of IL-1β present. (27)

Typical Results

The following results are shown for illustration only and should not be used to calculate results from another assay.

Well	Standard	Average OD	IL-1β (pg/mL)
A1,A2	S1	2.001	200
B1,B2	S2	1.051	100
C1,C2	S3	0.556	50
D1,D2	S4	0.290	25
E1,E2	S5	0.159	12.5
F1,F2	S6	0.088	6.25
G1,G2	S7	0.056	3.13
H1,H2	Zero	0.021	0

Salivary IL-1β Example Ranges*

Group	N	Mean (pg/mL)	Mean (x15 Dilution) (pg/mL)	Standard Deviation (pg/mL)	Mean adjusted for flow rate (pg/min.)	Correlation of Mean (x15 Dilution) to Flow Rate
Adults	27	8	122	138	72	-0.184

*To be used as a guide only. Each laboratory should establish its own range.

APPENDIX D: Instructions for Using the Supplement

Study: ***The effect of bovine colostrum supplementation on fitness, muscle mass, inflammation and immune function during intense training in rugby players.***

Directions for use

- Fully mix or blend 1 level scoop of powder into 4 to 8 oz. (1/2 to 1 cup) of smoothie, juice or water.
- Consume 3 level scoops of powder daily for 8 weeks.
- On days when you train, consume 1 scoop before your training session, 1 scoop after your training session, and 1 scoop with a meal. On days you don't train consume 1 scoop three times during the day with meals.

For any questions or concerns please call:

Phil @ 306-966-1072, OR 306-230-3849
Eliran @ 306-261-2345

APPENDIX E: Bovine Colostrum Product Information

SCCL
SCIENCE • NATURE • CARE

Manufacturer's Attestation of Ingredients

Product: Bovine Dried Colostrum

Trade name: Eterna

Serial: N.A.

Intended use: Eterna is a human supplement.

Manufacturer:
The Saskatoon Colostrum Company Ltd.
30 Molaro Place
Saskatoon, Saskatchewan, Canada
S7K 6A2

List of ingredients:
Medicinal:
Bovine Colostrum (*Bos Taurus*) – 98.1%
Non-medicinal:
Polysorbate 80 – 0.84%
Polyoxyethylene (3) Monooleate – 0.84%
Mixed tocopherols (non GMO sunflower oil sourced) – 0.22%

Other products this load may have come contact with:
This product does not come into contact with any other products or materials.

Whether animal protein products are used by this manufacturer at the facility that this entry originated from:
This product contains bovine colostrum (first milk). There are no other products of animal origin processed at the facilities used in the manufacture of this product.

Ron Sargent, Director of Quality Assurance Date

The Saskatoon Colostrum Company Ltd.
30 Molaro Place • Saskatoon, Saskatchewan • Canada S7K 6A2
Telephone: (306) 242-3185 or 1-866-242-3185 (Toll Free) • Facsimile: (306) 373-5766
www.saskcolostrum.com

4. Eterna™ Gold Athletic Powder

Unit Quantity Packaging: 360 g jar

Natural Product Number: EN-177413

Medicinal Ingredients:
Bovine Colostrum (*Bos Taurus*).....20 g per scoop
Non-Medicinal Ingredients:
Calcium silicate, polyoxyethylene (3) monooleate, polysorbate 80. This product does not contain synthetic hormones, antibiotics, artificial colour or flavour, wheat, corn, gluten, or preservatives.

Suggested Usage: Adults, mix 1 scoop (20 grams) of powder in 4 - 8 ounces of a smoothie, juice or water, or enjoy with one serving of yogurt or cereal. Take 1 -3 times daily. Allow 8 weeks of consecutive use for best results.

Description: Eterna™ Gold colostrum helps to improve athletic performance and increase lean body mass when used during athletic training.

Bovine colostrum helps to burn fat while increasing muscle mass when used in conjunction with exercise. It also helps to repair damaged muscle fibres in athletes, provides anti-inflammatory benefits, allows increased nutrient absorption and improves muscle tone, skin elasticity and increased bone density. Strenuous exercise, training and competition can compromise your immune system which can lead to infectious diseases; because colostrum supports your immune systems, it can help to reduce the risk of infection after exercise. Colostrum will help cut recovery time from hard workouts and can speed the healing of muscle, tendon and ligament injuries. Finally, athletes may suffer from 'leaky gut' due to protein supplementation–colostrum is known to help leaky gut.

Supports Immune System • Enhances Athletic Performance • Helps Burn Fat • Increases Lean Body Mass • Helps to Repair Damaged Muscle Fibres • Helps Reduce Recovery Time • Supports Gut Health

Caution: Contains Dairy. Do not use if you have a known allergy to dairy products. Consult a health care practitioner prior to use if you are pregnant or breastfeeding if you have an immune system disorder, if you have cancer or a family history of cancer, if you have diabetes, if you have a liver or kidney disease, or if you have been instructed to follow a low protein diet.

APPENDIX F: Statistical Tables

Body composition (DXA)

Tests of Within-Subjects Effects

Measure: Lean_Body_Mass

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	4.615	1	4.615	11.754	.003
	Greenhouse-Geisser	4.615	1.000	4.615	11.754	.003
	Huynh-Feldt	4.615	1.000	4.615	11.754	.003
	Lower-bound	4.615	1.000	4.615	11.754	.003
Time * group	Sphericity Assumed	.001	1	.001	.002	.964
	Greenhouse-Geisser	.001	1.000	.001	.002	.964
	Huynh-Feldt	.001	1.000	.001	.002	.964
	Lower-bound	.001	1.000	.001	.002	.964
Error(Time)	Sphericity Assumed	7.853	20	.393		
	Greenhouse-Geisser	7.853	20.000	.393		
	Huynh-Feldt	7.853	20.000	.393		
	Lower-bound	7.853	20.000	.393		

Tests of Within-Subjects Effects

Measure: Fat_Percentage

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	.000	1	.000	.000	.986
	Greenhouse-Geisser	.000	1.000	.000	.000	.986
	Huynh-Feldt	.000	1.000	.000	.000	.986
	Lower-bound	.000	1.000	.000	.000	.986
Time * group	Sphericity Assumed	.006	1	.006	.008	.929
	Greenhouse-Geisser	.006	1.000	.006	.008	.929
	Huynh-Feldt	.006	1.000	.006	.008	.929
	Lower-bound	.006	1.000	.006	.008	.929
Error(Time)	Sphericity Assumed	14.079	20	.704		
	Greenhouse-Geisser	14.079	20.000	.704		
	Huynh-Feldt	14.079	20.000	.704		
	Lower-bound	14.079	20.000	.704		

Tests of Within-Subjects Effects

Measure: Total Fat mass

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	.526	1	.526	.321	.578
	Greenhouse-Geisser	.526	1.000	.526	.321	.578
	Huynh-Feldt	.526	1.000	.526	.321	.578
	Lower-bound	.526	1.000	.526	.321	.578
Time * group	Sphericity Assumed	.226	1	.226	.138	.715
	Greenhouse-Geisser	.226	1.000	.226	.138	.715
	Huynh-Feldt	.226	1.000	.226	.138	.715
	Lower-bound	.226	1.000	.226	.138	.715
Error(Time)	Sphericity Assumed	32.802	20	1.640		
	Greenhouse-Geisser	32.802	20.000	1.640		
	Huynh-Feldt	32.802	20.000	1.640		
	Lower-bound	32.802	20.000	1.640		

Tests of Within-Subjects Contrasts

Source	Measure	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Lean_Tissue_Mass_Arms	Linear	.191	1	.191	.261	.615
	Lean_Tissue_Mass_Trunk	Linear	11.506	1	11.506	5.349	.031
	Lean_Tissue_Mass_Legs	Linear	.001	1	.001	.007	.935
Time * group	Lean_Tissue_Mass_Arms	Linear	.051	1	.051	.070	.794
	Lean_Tissue_Mass_Trunk	Linear	.902	1	.902	.419	.525
	Lean_Tissue_Mass_Legs	Linear	.114	1	.114	.595	.450
Error(Time)	Lean_Tissue_Mass_Arms	Linear	14.643	20	.732		
	Lean_Tissue_Mass_Trunk	Linear	43.017	20	2.151		
	Lean_Tissue_Mass_Legs	Linear	3.834	20	.192		

Tests of Within-Subjects Effects

Measure: Weight

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	8.282	1	8.282	3.251	.086
	Greenhouse-Geisser	8.282	1.000	8.282	3.251	.086
	Huynh-Feldt	8.282	1.000	8.282	3.251	.086
	Lower-bound	8.282	1.000	8.282	3.251	.086
Time * Group	Sphericity Assumed	.021	1	.021	.008	.929
	Greenhouse-Geisser	.021	1.000	.021	.008	.929
	Huynh-Feldt	.021	1.000	.021	.008	.929
	Lower-bound	.021	1.000	.021	.008	.929
Error(Time)	Sphericity Assumed	50.947	20	2.547		
	Greenhouse-Geisser	50.947	20.000	2.547		
	Huynh-Feldt	50.947	20.000	2.547		
	Lower-bound	50.947	20.000	2.547		

Muscle Thickness (U.S)

Tests of Within-Subjects Effects

Measure: Mean_Arm_Thickness

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	.378	1	.378	14.434	.001
	Greenhouse-Geisser	.378	1.000	.378	14.434	.001
	Huynh-Feldt	.378	1.000	.378	14.434	.001
	Lower-bound	.378	1.000	.378	14.434	.001
Time * group	Sphericity Assumed	.005	1	.005	.206	.655
	Greenhouse-Geisser	.005	1.000	.005	.206	.655
	Huynh-Feldt	.005	1.000	.005	.206	.655
	Lower-bound	.005	1.000	.005	.206	.655
Error(Time)	Sphericity Assumed	.524	20	.026		
	Greenhouse-Geisser	.524	20.000	.026		
	Huynh-Feldt	.524	20.000	.026		
	Lower-bound	.524	20.000	.026		

Tests of Within-Subjects Effects

Measure: Mean_Leg_Thickness

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	.360	1	.360	1.711	.206
	Greenhouse-Geisser	.360	1.000	.360	1.711	.206
	Huynh-Feldt	.360	1.000	.360	1.711	.206
	Lower-bound	.360	1.000	.360	1.711	.206
Time * group	Sphericity Assumed	.177	1	.177	.842	.370
	Greenhouse-Geisser	.177	1.000	.177	.842	.370
	Huynh-Feldt	.177	1.000	.177	.842	.370
	Lower-bound	.177	1.000	.177	.842	.370
Error(Time)	Sphericity Assumed	4.210	20	.210		
	Greenhouse-Geisser	4.210	20.000	.210		
	Huynh-Feldt	4.210	20.000	.210		
	Lower-bound	4.210	20.000	.210		

Strength

Tests of Within-Subjects Effects

Measure: Bench_Press

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	820.455	1	820.455	2.012	.171
	Greenhouse-Geisser	820.455	1.000	820.455	2.012	.171
	Huynh-Feldt	820.455	1.000	820.455	2.012	.171
	Lower-bound	820.455	1.000	820.455	2.012	.171
Time * group	Sphericity Assumed	275.000	1	275.000	.674	.421
	Greenhouse-Geisser	275.000	1.000	275.000	.674	.421
	Huynh-Feldt	275.000	1.000	275.000	.674	.421
	Lower-bound	275.000	1.000	275.000	.674	.421
Error(Time)	Sphericity Assumed	8154.545	20	407.727		
	Greenhouse-Geisser	8154.545	20.000	407.727		
	Huynh-Feldt	8154.545	20.000	407.727		
	Lower-bound	8154.545	20.000	407.727		

Tests of Within-Subjects Effects

Measure: Leg_Press

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	4085.818	1	4085.818	1.411	.249
	Greenhouse-Geisser	4085.818	1.000	4085.818	1.411	.249
	Huynh-Feldt	4085.818	1.000	4085.818	1.411	.249
	Lower-bound	4085.818	1.000	4085.818	1.411	.249
Time * group	Sphericity Assumed	1040.818	1	1040.818	.360	.556
	Greenhouse-Geisser	1040.818	1.000	1040.818	.360	.556
	Huynh-Feldt	1040.818	1.000	1040.818	.360	.556
	Lower-bound	1040.818	1.000	1040.818	.360	.556
Error(Time)	Sphericity Assumed	57902.364	20	2895.118		
	Greenhouse-Geisser	57902.364	20.000	2895.118		
	Huynh-Feldt	57902.364	20.000	2895.118		
	Lower-bound	57902.364	20.000	2895.118		

Vertical Jump

ANOVA:

Tests of Within-Subjects Effects

Measure: VJ

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
time	Sphericity Assumed	2.500	1	2.500	10.588	.004	.370
	Greenhouse-Geisser	2.500	1.000	2.500	10.588	.004	.370
	Huynh-Feldt	2.500	1.000	2.500	10.588	.004	.370
	Lower-bound	2.500	1.000	2.500	10.588	.004	.370
time * Group	Sphericity Assumed	2.500	1	2.500	10.588	.004	.370
	Greenhouse-Geisser	2.500	1.000	2.500	10.588	.004	.370
	Huynh-Feldt	2.500	1.000	2.500	10.588	.004	.370
	Lower-bound	2.500	1.000	2.500	10.588	.004	.370
Error(time)	Sphericity Assumed	4.250	18	.236			
	Greenhouse-Geisser	4.250	18.000	.236			
	Huynh-Feldt	4.250	18.000	.236			
	Lower-bound	4.250	18.000	.236			

Tests of Within-Subjects Contrasts

Measure: VJ

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
time	Linear	2.500	1	2.500	10.588	.004	.370
time * Group	Linear	2.500	1	2.500	10.588	.004	.370
Error(time)	Linear	4.250	18	.236			

Post-hoc:

T-Test for Soy

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Vertical_Jump_PRE	18.1000	10	3.61939	1.14455
	Vertical_Jump_POST	18.1000	10	3.54181	1.12002

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Vertical_Jump_PRE & Vertical_Jump_POST	10	.985	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Vertical_Jump_PRE - Vertical_Jump_POST	.00000	.62361	.19720	-.44610	.44610	.000	9	1.000

T-Test for BC

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Vertical_Jump_PRE	16.1000	10	3.68028	1.16381
	Vertical_Jump_POST	17.1000	10	3.52609	1.11505

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Vertical_Jump_PRE & Vertical_Jump_POST	10	.980	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Vertical_Jump_PRE - Vertical_Jump_POST	-1.00000	.74536	.23570	-1.53320	-.46680	-4.243	9	.002

ANCOVA:

Tests of Between-Subjects Effects

Dependent Variable: Vertical_Jump_PRE

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	20.000[a]	1	20.000	1.501	.236
Intercept	5848.200	1	5848.200	438.981	.000
Group	20.000	1	20.000	1.501	**.236**
Error	239.800	18	13.322		
Total	6108.000	20			
Corrected Total	259.800	19			

Tests of Between-Subjects Effects

Dependent Variable: Vertical_Jump_POST

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	221.915[a]	3	73.972	150.094	.000
Intercept	1.368	1	1.368	2.776	.115
Group	.351	1	.351	.712	.411
Vertical_Jump_PRE	216.912	1	216.912	440.132	.000
Group * Vertical_Jump_PRE	.039	1	.039	.079	***.782***
Error	7.885	16	.493		
Total	6425.000	20			
Corrected Total	229.800	19			

Tests of Between-Subjects Effects

Dependent Variable: Vertical_Jump_POST

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	221.876[a]	2	110.938	237.996	.000	.966
Intercept	1.410	1	1.410	3.025	.100	.151
Vertical_Jump_PRE	216.876	1	216.876	465.266	.000	.965
Group	3.755	1	3.755	8.055	**.011**	**.322**
Error	7.924	17	.466			
Total	6425.000	20				
Corrected Total	229.800	19				

a. R Squared = .966 (Adjusted R Squared = .961)

Aerobic Fitness

Tests of Within-Subjects Effects

Measure: vo2max

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
time	Sphericity Assumed	37.588	1	37.588	5.796	.026	.234
	Greenhouse-Geisser	37.588	1.000	37.588	5.796	.026	.234
	Huynh-Feldt	37.588	1.000	37.588	5.796	.026	.234
	Lower-bound	37.588	1.000	37.588	5.796	.026	.234
time * group	Sphericity Assumed	16.574	1	16.574	2.556	.126	.119
	Greenhouse-Geisser	16.574	1.000	16.574	2.556	.126	.119
	Huynh-Feldt	16.574	1.000	16.574	2.556	.126	.119
	Lower-bound	16.574	1.000	16.574	2.556	.126	.119
Error(time)	Sphericity Assumed	123.226	19	6.486			
	Greenhouse-Geisser	123.226	19.000	6.486			
	Huynh-Feldt	123.226	19.000	6.486			
	Lower-bound	123.226	19.000	6.486			

Tests of Within-Subjects Contrasts

Measure: vo2max

Source	time	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
time	Linear	37.588	1	37.588	5.796	.026	.234
time * group	Linear	16.574	1	16.574	2.556	.126	.119
Error(time)	Linear	123.226	19	6.486			

Markers of Immune Function

Tests of Within-Subjects Effects

Measure: CRP

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	50252050.187	1	50252050.187	7.108	.015
	Greenhouse-Geisser	50252050.187	1.000	50252050.187	7.108	.015
	Huynh-Feldt	50252050.187	1.000	50252050.187	7.108	.015
	Lower-bound	50252050.187	1.000	50252050.187	7.108	.015
Time * group	Sphericity Assumed	16982697.508	1	16982697.508	2.402	.137
	Greenhouse-Geisser	16982697.508	1.000	16982697.508	2.402	.137
	Huynh-Feldt	16982697.508	1.000	16982697.508	2.402	.137
	Lower-bound	16982697.508	1.000	16982697.508	2.402	.137
Error(Time)	Sphericity Assumed	141401672.867	20	7070083.643		
	Greenhouse-Geisser	141401672.867	20.000	7070083.643		
	Huynh-Feldt	141401672.867	20.000	7070083.643		
	Lower-bound	141401672.867	20.000	7070083.643		

Non parametric Mann-Whitney U-test

	Rank Sum Group 1	Rank Sum Group 2	U	Z	p-level	Z adjusted	p-level	Valid N Group 1	Valid N Group 2	2*1sided exact p
change	126.0000	127.0000	48.00000	-0.791257	0.428795	-0.791257	0.428795	12	10	0.456151

Tests of Within-Subjects Effects

Measure: IgA

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	4052.564	1	4052.564	.096	.760
	Greenhouse-Geisser	4052.564	1.000	4052.564	.096	.760
	Huynh-Feldt	4052.564	1.000	4052.564	.096	.760
	Lower-bound	4052.564	1.000	4052.564	.096	.760
Time * group	Sphericity Assumed	50019.911	1	50019.911	1.181	.290
	Greenhouse-Geisser	50019.911	1.000	50019.911	1.181	.290
	Huynh-Feldt	50019.911	1.000	50019.911	1.181	.290
	Lower-bound	50019.911	1.000	50019.911	1.181	.290
Error(Time)	Sphericity Assumed	847415.432	20	42370.772		
	Greenhouse-Geisser	847415.432	20.000	42370.772		
	Huynh-Feldt	847415.432	20.000	42370.772		
	Lower-bound	847415.432	20.000	42370.772		

Tests of Within-Subjects Effects

Measure: IL1b

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	95594.220	1	95594.220	.720	.406
	Greenhouse-Geisser	95594.220	1.000	95594.220	.720	.406
	Huynh-Feldt	95594.220	1.000	95594.220	.720	.406
	Lower-bound	95594.220	1.000	95594.220	.720	.406
Time * group	Sphericity Assumed	5029.453	1	5029.453	.038	.848
	Greenhouse-Geisser	5029.453	1.000	5029.453	.038	.848
	Huynh-Feldt	5029.453	1.000	5029.453	.038	.848
	Lower-bound	5029.453	1.000	5029.453	.038	.848
Error(Time)	Sphericity Assumed	2657108.396	20	132855.420		
	Greenhouse-Geisser	2657108.396	20.000	132855.420		
	Huynh-Feldt	2657108.396	20.000	132855.420		
	Lower-bound	2657108.396	20.000	132855.420		

Tests of Within-Subjects Effects

Measure: IL6

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Sphericity Assumed	.625	1	.625	.176	.679
	Greenhouse-Geisser	.625	1.000	.625	.176	.679
	Huynh-Feldt	.625	1.000	.625	.176	.679
	Lower-bound	.625	1.000	.625	.176	.679
Time * group	Sphericity Assumed	.133	1	.133	.037	.848
	Greenhouse-Geisser	.133	1.000	.133	.037	.848
	Huynh-Feldt	.133	1.000	.133	.037	.848
	Lower-bound	.133	1.000	.133	.037	.848
Error(Time)	Sphericity Assumed	70.920	20	3.546		
	Greenhouse-Geisser	70.920	20.000	3.546		
	Huynh-Feldt	70.920	20.000	3.546		
	Lower-bound	70.920	20.000	3.546		

Energy Intake

Tests of Within-Subjects Contrasts

Source	Measure	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Calories	Linear	11155.600	1	11155.600	.431	.520
	Protein	Linear	50.625	1	50.625	.548	.469
	CHO	Linear	330.625	1	330.625	.599	.449
	FAT	Linear	46.225	1	46.225	.376	.547
Time * Group	Calories	Linear	3.600	1	3.600	.000	.991
	Protein	Linear	112.225	1	112.225	1.216	.285
	CHO	Linear	342.225	1	342.225	.620	.441
	FAT	Linear	11.025	1	11.025	.090	.768
Error(Time)	Calories	Linear	466014.800	18	25889.711		
	Protein	Linear	1661.650	18	92.314		
	CHO	Linear	9933.650	18	551.869		
	FAT	Linear	2213.250	18	122.958		